

ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL CANCER INSTITUTE

FISCAL YEAR 1973

PART II

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

NATIONAL CANCER INSTITUTE

ANNUAL REPORT

July 1, 1972 through June 30, 1973

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SUMMARY REPORT
OF THE
DIRECTOR
DIVISION OF CANCER BIOLOGY AND DIAGNOSIS
NATIONAL CANCER INSTITUTE

July 1, 1972 through June 30, 1973

The Division of Cancer Biology and Diagnosis was established in August, 1972, to give better visibility to the two major directions in cancer research of the division's predecessor, General Laboratories and Clinics. In addition to major efforts in cancer biology and in cancer diagnosis the division supports smaller, but still sizeable research efforts in cancer causation and in certain restricted aspects of cancer treatment.

The division's research program may be divided in three broad areas: cancer biology, immunology, and clinical research. The organizational structure roughly follows this same division: first, a group of laboratories performing basic research in cancer biology immediately under the Director; second, two elements, the Immunology Branch and the Laboratory of Cell Biology, working in the immunobiology, immunodiagnosis and immunotherapy of cancer, assigned together with a sizeable contract program to an Associate Director for Immunology; and third, clinical branches working intramurally in the fields of surgery, radiation, dermatology, metabolism and pathology under an Associate Director for Clinical Research. Clinical research includes both cancer diagnosis and treatment. A fourth organizational element, the office of the Associate Director for Program Planning, performs certain broad administrative functions but also for convenience includes the monitorship of two major contract programs, those in cancer diagnosis and in breast cancer. There is considerable crossover of research directions between the various research laboratories and clinical branches, and also between major program divisions.

Several organizational changes were made during the year. These include the establishment of the positions and offices of the Associate Director for Immunology and of the Associate Director for Program Planning. A new Laboratory of Pathophysiology was established from the former Tumor Pathophysiology Section of the Laboratory of Biochemistry. A new Diagnosis Branch was created to handle the growing contract program in the field of cancer diagnosis. The former Endocrine Evaluation Branch was renamed the Breast Cancer Program Coordinating Branch.

CANCER BIOLOGY

In the Laboratory of Biochemistry progress has been made in the understanding of various aspects of leucocyte maturation. Pronounced differences in glycoprotein patterns have been found, suggesting that many new glycoproteins appear in the later stages of neutrophil maturation. Nearly complete separation of the granules of guinea pig polymorphonuclear leucocytes has been achieved; granule proteins are now being characterized.

It was reported last year that incubation of mouse cells deficient in a certain enzyme with isolated hamster chromosomes produced growing cells with the enzyme activity. By further study the enzyme, hypoxanthine-guanine phosphoribosyl transferase, has been shown by physical characterization to be of the donor, hamster, type in 10 of 11 separate clones examined. Further evidence of this apparent uptake of chromosomes by cells is proceeding by analysis of the clones for another enzyme, adenine phosphoribosyl transferase, missing in the mouse cells, but present on another hamster chromosome.

Further work has been done with a distinctive DNA polymerase from A particles, virus like structures found in certain tumors, mainly in mice. In both myeloma and neuroblastoma cells the enzyme activity appears to be a specific marker for the intracisternal A particle. Systemic fractionation of mouse myeloma was undertaken to define the types of DNA synthesizing enzymes present. Multiple forms of DNA polymerase activity were distinguished, of which three corresponded with previously recognized activities. Three other forms were maximally or exclusively active with a synthetic template, polyriboadenylate, of which one seems to be the A particle enzyme. The functional roles of these in gene replication and repair will be studied further in detail.

In the Laboratory of Biology, tissue culture studies have been aided by the development of a pulsatile perfusion system for cell culture and of a medium containing an autoclarable serum substitute. In studies of spontaneous neoplastic transformation of cells *in vitro* it has been found that cultured fetal cells lose fetal antigens in the process. A low molecular weight horse serum fraction accelerates transformation, and components of fetal bovine serum inhibit it. Study of aberrant nucleic acid methylation as a causative factor in transformation was aided by the development of a very sensitive assay method for methylated bases, which has shown an increase in neoplastic versus non-neoplastic cell lines. Nucleic acid methylase activity is also under study in such cell lines and in oncogenic viruses.

Work on mammary tumorigenesis in the mouse has included the purification of intracytoplasmic A particles, thought to be an immature form the B particle, the mammary tumor virus. Cross reacting antigens have been found in the two particles. Various lines of mammary tumor virus have been found to differ in capacity to induce tumors, and in transmission. The NIV strain appears to be transmitted genetically as a provirus. A genetic

analysis, including location, is in progress with a mouse strain that is very highly susceptible to tumor formation, and with immunologic test of the NIV antigen. This work should provide a very useful model for the human situation, since if there is a breast cancer virus in women it is probably transmitted genetically.

A major project of the Laboratory of Molecular Biology is the study of the role of cyclic AMP in malignant transformation of cells. A transforming gene may act through control of the level of a regulatory molecule, i.e., cyclic AMP. Many transformed cells have low levels of cyclic AMP. The reasons for lowering are complex. In some cases the synthetic enzyme is reduced; its inactivation proceeds in different ways with different strains of transforming virus. Sometimes the degradative enzyme is increased, or cyclic AMP is lost from the cells. Treatment of transformed cells with agents that raise cyclic AMP restores many properties toward normal, including cell shape, adhesiveness and motility. Contractile proteins may be involved; myosin and actin have now been found in fibroblasts. The interaction of cyclic AMP with these molecules is now under study in normal and transformed cells.

Much effort is devoted to studies of the control of gene expression in the transcription of certain specific operons of *E. coli* DNA. Cyclic AMP first combines with a receptor protein (CRP); several further steps must occur before RNA polymerase can begin to transcribe the gal operon. Cyclic AMP and CRP are also required for transcription of the lac operon. This process is stimulated by an unusual nucleotide which has been found to need tRNA to act; this is a noteworthy link between transcription and translation.

Valuable insights into mitogenesis by viruses are coming from studies of the integration of the bacterial virus, lambda, into the *E. coli* chromosome, and its later excision. When the virus is integrated in unusual sites it acts as a mitogen, inactivating a variety of different genes. Further genetic damage may occur on virus excision by removal of neighboring bacterial genes. Other work analyzing how lambda decides to lysogenize a cell, rather than kill it is directly related to the problem of malignant transformation of cells by viruses. It has been found that regulatory proteins act at a single site on the lambda chromosome, both to activate transcription of gene products favoring lysogeny and to inhibit synthesis of products favoring cell death.

In the Laboratory of Physiology study of the fine structure of the thyroid gland concentrated on pinocytosis of colloid from the lumen of the follicle. This takes place by the insertion of a sheet of cytoplasm which envelops colloid and draws it back into the cell. The process is rapid and can be completed in 10 minutes. Study of the regulation of energy and water exchange in whole rats with large growing tumors indicate that limitation of motor activity partly causes the hypophagia of cancer. Feeding response to hypoglycemia and cold are normal. The evidence suggests that tumor growth causes no essential change in the regulation of food and water intake, but that there is active behavioral regulation to attempt to compensate for the redistribution of energy flow.

The control of macromolecule synthesis, an aspect of the cell's protection against virus infection, is under study in two areas: DNA synthesis and degradation in *E. coli* after withdrawal of methionine, and the kinetics of the interconversion of pseudouridine and uridine. Collagen synthesis is under investigation in chick embryos and normal and transformed fibroblasts. Ascorbic acid stimulates collagen secretion in normal cells, but transformed cells are much less dependent on the vitamin for maximal secretion.

In radiation biology, molecular studies of the effect of metal ions on reducing free radical production from radiated DNA suggests that the ions act by quenching excited states and by charge scavenging. Another aspect of research in radiation biology involves immunogenetic study of bone marrow transplantation, a valuable adjunct to cancer treatment if GVH reaction can be avoided. The observation in rats that mother's marrow caused relatively few reactions in radiated progeny, daughters being more tolerant than sons, was amplified by studies of transplantation of fertilized ova of one strain into foster mothers of another. Antigen responsiveness of the fetus, and reactivity as well, was reduced by maternal influences, but in one case involving the H-Z and Y antigens of male mice, there was no reduction, but rather an increase in reactivity.

The major programs of the newly established Laboratory of Pathophysiology is directed toward the understanding of the regression mechanism in hormone dependent mammary tumors. In rat mammary tumors, the vascular, interstitial and cellular compartments were similar in growing and regressing tumors on a tissue weight basis, as were blood supply and metabolite consumption. Increased lysosomal enzyme activity was confined to the cells. One such enzyme is ribonuclease which is rapidly synthesized after hormone withdrawal; its degradation is also increased. Lysosomal enzyme activity can be blocked by cycloheximide, but tumor regression continues unchanged. It is believed that lysosomal enzymes are not involved in cell destruction, but only in cannibalism of cell fragments.

An artificial capillary network has been developed which will sustain the growth of tumor cells at densities comparable to those of a tumor in vivo. In this system choriocarcinoma cells produced ten times as much gonadotropin per cell as in monolayer culture. Studies of mammary tumor regression are being started with this system. In other work assay methods for tumor angiogenesis factor are being developed. It is hoped to be able to purify this factor, with the eventual goal of developing an anti angiogenesis factor that might inhibit tumor growth and metastasis.

The Laboratory of Theoretical Biology has now been in operation for one year as part of the Division of Cancer Biology and Diagnosis, continuing, with additions, research efforts of some years formerly supported in another institute of NIH. Work involves three areas, theoretical biology, computations, and modeling. The major project in theoretical biology is the development of theory for membrane transport to account for or apply to cellular control processes at the membrane level. When non-linear transport processes, e.g., conformation changes in membrane transport

proteins, are coupled to enzymatic degradation on lateral diffusion, the models exhibit interesting dynamic patterns such as multiple steady state transitions, oscillations, spatial patterns and propagation of chemical waves. In the area of computation, further development continues on the SAAM program (a huge program of some 30,000 FORTRAN statements in about 200 subroutines) a general purpose computer program for biological research, embodying many years of experience in mathematical modeling and computation. In the area of modeling, the staff is collaborating with many investigators in NCI and elsewhere to develop mathematical models of various kinetic biologic systems.

IMMUNOLOGY

The rapid development of the field of immunology in recent years has had tremendous application in cancer research. Tumor immunology research comprises the greatest part of the program of three laboratories in the Division of Cancer Biology and Diagnosis. Two of these, the Immunology Branch and the Laboratory of Cell Biology are assigned organizationally under the Associate Director for Immunology. Although the third, the Metabolism Branch, is under the Associate Director for Clinical Research, its program will be summarized in this section. The growing tumor immunology contract program is included in the contract section of this report.

A large part of the research of the Immunology Branch involves the structure and function of cell membranes of immune cells and tumor cells. Membranes containing **histocompatibility** antigens have been solubilized while retaining antigenic activity. Such antigen density is found to vary during the cell cycle. After treatment with neuraminidase lymphoid cell surfaces react with an antibody in normal human serum; this finding may be of value in working out how neuraminidase increases tumor cell immunogenicity. Antisera have been developed to distinguish human T and B lymphoid cells. Cell surface receptors for human IgG have proved to be reliable markers for B cells. Receptors for mitogens, insulin and growth hormone have also been isolated. Follow on study of the leukemia specific antigen reported earlier reveal a similar antigen on human, but not mouse, cells infected with murine leukemia virus. Work is in progress to raise antisera to human tumor cells, especially CLL cells where parental cells can be used for cross absorption, and in multiple myeloma.

Research on cellular immunity is directed toward identifying the characteristics of lymphocytes involved in both recognition and effector reactions. Lymphoid cells that suppress cell mediated immunity appear to exist, and probably come from the adult thymus. A lymphocyte dependent antibody, an IgG, has been identified which coats target cells and sensitizes them for destruction by nonimmune lymphoid cells, probably B cells. Cell mediated cytotoxicity has been shown to be a function of lymphoid cells with histamine receptors.

In the area of genetic control of the immune response, it has been shown in rodents that such control exists of antibody formation to

staphylococcal nuclease. In mice, cell mediated immunity is also under such control. With purified mRNA from mouse myeloma cells, production of part of the specific immunoglobulin has been accomplished in a cell free system. It is planned to use such mRNA in hybridization experiments to estimate the number of immunoglobulin genes in somatic cells.

Since carcinofetal antigens have shown the most usefulness to date in immunodiagnosis, structural studies of alpha fetoprotein and of carcinoembryonic antigen have been undertaken. Research in immunotherapy includes animal studies of the treatment of tumors with BCG, "deblocking sera," and by neuraminidase treated cells. Studies of these materials are also in progress in carefully selected patients in collaboration with a number of clinical groups at NIH.

A large part of the research of the Laboratory of Cell Biology centers on tumor associated antigens. A quantitative method has been developed for factors which "block" cell mediated immunity. Antisera from humorally tolerant or completely tolerant mice have no blocking activity. Preliminary data suggest that blocking can be caused by a complex of IgG and H-Z antigen. VSTA's have been solubilized and purified from dissociated cells of two different neoplasms, RBL-5 and mKSA. The purified antigens will immunize syngeneic mice against the specific and antigenically related tumors.

Work in the immunodiagnosis of human tumors has led to the development of an assay for alpha fetoprotein sensitive enough to measure normal levels in serum. Serums from cancer patients showed greater than normal levels in embryonal cell cancer, lung cancer, pancreatic cancer, gastric cancer (in decreasing order of fraction positive). A very few colon cancers were positive, but no esophageal or breast cancer serums were positive. Extensive in vitro assays are being performed to measure the tumor associated antigens of human cancers; the leukocytes of cancer patients in migration assay are inhibited by some extracts of tumors of the same histologic type. The skin reactivity of cancer patients is being tested with antigens from various neoplasms: solubilized fractionated antigens from AML cells are reactive; the skin reactive antigen of intestinal cancer is found in several CEA preparations but is distinct from the CEA of Gold; skin reactive antigens from breast and lung cancer, and from melanoma are not distinguishable from such antigens from normal tissue.

In immunochemical studies of mouse myeloma proteins, the molecular structure of the antibody combining site has been determined by x-ray crystallography in a pepsin fragment of a specific phosphorylcholine binding myeloma protein. Amino acid sequence analysis of the various claims of this and other myeloma proteins is continuing. Other work in the Laboratory of Cell Biology is directed toward the isolation of viruses from various animal tumors, research on ganglioside biosynthesis in cells with virus transformation, and cell hybridization work with emphasis on cell surface antigens and their specification, and host specificity of oncogenic viruses.

The research program of the Metabolism Branch is primarily clinically oriented, and is directed toward to major goals. The first is to define host factors that result in a high incidence of neoplasia; the second is to determine the physiological and biochemical effects that a tumor produces on the metabolism of the host. Patients with three immuno deficiency diseases and a high incidence of neoplasia are under study both to find ways to repair their immunological defects and to gain insight into the action of the immune surveillance mechanism in man. In the disease ataxia telangiectasia there is a defect in the ability to synthesize immunoglobulins and lymphocytes, the effectors of the immune system. In the Wiskott-Aldrich syndrome defects exist in antigen processing and recognition, the afferent limb of immunity. In the third situation, of which intestinal lymphangiectasia is an example, there is short survival of immunoglobulins and lymphocytes.

It has recently been found that patients with ataxia telangiectasia have very high levels of alpha fetoprotein; this supports the hypothesis that the basic defect is an abnormality in tissue differentiation due to imperfect interaction between entodermal and mesodermal elements in early development. Defects in lymphocyte response to mitogens and in target cell killing have also been noted in patients with chronic lymphatic leukemia. The predominant lymphocyte was a B cell which synthesized IgM in vitro, but not other immunoglobulins. The disorder appears to be a malignancy of a single clone of B cells.

Patients with the Wiskott-Aldrich syndrome have normal numbers of circulating lymphocytes, which respond to mitogens and which have the killer function intact. Immunoglobulin production is normal. In spite of this normal functioning of the afferent limb of immunity, there is a defect in the afferent limb.

In intestinal lymphangiectasia serum proteins and lymphocytes are lost into the gastrointestinal tract. There is a depletion of circulating lymphocytes and depressed cellular immunity. This appears to be caused by selective loss of T cells, for among circulating lymphocytes the fraction bearing immunoglobins on their surfaces (i.e., B cells) is much increased.

Intestinal biopsy specimens from patients with active gluten sensitive enteropathy were found to synthesize alkaline phosphatase normally in vitro, unless gliadin was added. This repression did not occur in biopsies from patients in remission when they were cultured individually with gliadin, but if a biopsy from a patient with active disease was added to the culture, the production of alkaline phosphatase ceased. This is good evidence that an endogenous mechanism, such as the immune system, must be activated for gliadin to be toxic.

CLINICAL RESEARCH

The area of clinical research includes cancer surgery and radiotherapy, work on skin cancer, and general cancer diagnosis. It is convenient to follow the organization by including here the programs of the various branches under the Associate Director for Clinical Research, although the research of these branches is much broader than just cancer diagnosis and treatment. The branches covered here are Surgery, Radiation, Dermatology and the Laboratory of Pathology. The program of the Metabolism Branch was reported under Immunology, and the Diagnosis contract program is included with Contract programs.

The Surgery Branch carries out active clinical and laboratory research programs, but the staff is also heavily involved in consultative and operative assistance to all of the intramural elements of the National Institutes of Health. The branch has ongoing clinical research in cancer of four areas, pelvic soft tissues, head and neck area, soft tissue and bone sarcomas, and melanomas. In head and neck cancer, encouraging results are being obtained with the addition of adjuvant methotrexate therapy. New surgical techniques are under development both for tumor removal and for reconstruction of defects. All new sarcoma patients are entering an anticoagulant study. Although there has been some postoperative morbidity, preliminary results suggest there may be some reduction in the propensity to metastasis of these tumors. A new Breast Oncology service is being established in cooperation with the medical staff of the Division of Cancer Treatment to do clinical research in all aspects of breast cancer.

The branch supports considerable clinical and laboratory research in tumor immunology. Clinical studies in cancer patients include the evaluation of in vitro lymphocyte reactivity to phytohemagglutinin(PHA) and contact sensitivity to dinitrochlorobenzene (DCNB). PHA reactivity preoperatively tended to be low; patients who were cured by operation seemed to be somewhat higher than in those not cured. Activity in patients with squamous carcinomas, sarcomas and melanomas was reduced, but those with adenocarcinomas showed no defects. Cancer patients were also generally less reactive than normals to DCNB, particularly patients with squamous carcinomas. There was a good correlation between these two test results. Additionally, HLA profiles have been determined in a series of patients with cancer of various types. It was found that HLA-B was more than twice as common in patients with squamous cancer than in normals or patients with other cancers. Monocyte function, part of the afferent limb of immunity, studied by latex particle phagocytosis and tetrazolium reduction, was also found to be defective in many cancer patients.

Results of laboratory research include a demonstration in mice that BCG vaccination reduces the incidence of melanomas after inoculation of tumor cells. In guinea pigs surgical interference with afferent lymphatic drainage, or removal of lymph nodes, did not prevent rejection of tumor allograft; systemic sensitization of the host eventually occurred. These findings

suggest that once a tumor is large enough to be detected clinically, a patient is already sensitized to it and will not benefit from leaving local lymph nodes intact, especially since many will contain metastatic tumor.

The program of the Radiation Branch is devoted entirely to studies in patients. The follow-up continues of patients with Stage I and II Hodgkin's disease treated with extensive radiation. The excellent survival has been maintained, being in excess of 90% at six years. The study of radiotherapy of other malignant lymphomas in cooperation with the Medicine Branch of the Division of Cancer Therapy has added many patients during the year.

A project in the multisystem therapy of Ewing's sarcoma has dominated the research of the Branch during the past year. This involves a systematic approach to integrated therapy, with careful documentation of the natural history of the disease, accurate location of any patient in the disease spectrum and selection of complementary approaches to treatment. Sixty patients with the disease have now been studied intensively. There has been a marked increase in survival. One-half of the patients with localized disease have survived three years, many apparently disease free, and one-third of those preventing with metastatic disease have survived for the same period. A similar multi-system project in the treatment of lung cancer was initiated during the year, but patient accrual has been slow because of lack of beds and clinical support, but hopefully work can be expanded in the coming year.

The Dermatology Branch supports both clinical and laboratory research projects in skin diseases with special emphasis on cancer. In patients with xeroderma pigmentosum, DNA repair has been studied on lymphocytes from peripheral blood. The amount of DNA repair is normal, but the rate of repair is slow. Fusion studies of cells from pairs of xeroderma pigmentosum patients show that there are at least four loci at which mutations can produce the repair defect. In other work the production of melanin is under study in black mice and in melanomas. Melanosomes from normal black mice contain some seven different proteins; the proteins of mouse melanoma melanosomes are quite different. These studies are being extended to human material.

Interesting information has come from study of the Sezary syndrome, a disease characterized by exfoliative skin disease and chronic lymphatic leukemia, plus certain large circulating, Sezary cells. These cells appear to be altered lymphocytes, since they react to PHA. DNA assays and chromosome counts show that they are tetraploid, although some smaller variants are diploid. These cells are not B lymphocytes, for they have no surface immunoglobulins. In a patient with the small cell variant, the cells react with human anti-T antibody. The disease thus appears to be a T lymphocyte leukemia (except for this disease, chronic lymphatic leukemia is a B cell disease). The identification of a T lymphocyte leukemia has important implications, since the T cell is involved in immunological surveillance against neoplasia.

Other studies include an evaluation of DCND immunotherapy of basal cell carcinomas; results were no better than with an irritant, croton oil.

Topical chemotherapy with BCNU is effective in the disease, at least when inflammation developed during treatment. CCNU has been able to produce long remissions in the treatment of psoriasis, but bone marrow depression sometimes occurred.

Although the Laboratory of Pathology is heavily involved in the training of residents in anatomic pathology, and in service histopathology and cytopathology for patients throughout the clinical center, it also carries out a broad research program with major emphasis in viral oncology and other aspects of carcinogenesis. Noteworthy research results include studies of the production of antibodies during oncogenesis in monkeys after Herpes virus saimiri infection. Animals without tumors show antibodies only to the late antigens, but those with tumors show antibodies to both early and late antigens. There are many similarities of this infection in monkeys to EB virus infection in man.

In another project a cell line established from a lung tumor has been shown to produce gonadotropin in vitro. In mouse cells a mixed infection has been established of arbo virus and murine leukemia virus. Arbo virus replication does not affect the budding and release of the murine leukemia virus. Detailed studies have been completed of the extent of cytomegalo virus infections in children with leukemia.

For the first time it has been possible to produce lung cancer experimentally from cigarette smoke; smoke condensate was mixed in wax pellets and implanted directly into the lungs of rats. Further, malignant mesotheliomas have now been induced in rats by asbestos and fibrous glass. Induction varies with different fiber size. These findings have great importance in public health.

CONTRACT PROGRAMS

The Division of Cancer Biology and Diagnosis supports several growing programs of contract research. The Tumor Immunology contract program is managed by the office of the Associate Director of Immunology. The program is divided into three parts, immunobiology, cancer immunodiagnosis, and cancer immunotherapy. The contract program of the Breast Cancer Task Force is managed by the Breast Cancer Program Coordinating Branch. This broad program is directed toward many different aspects of this very common cancer. The program includes four parts, epidemiology, experimental biology, diagnosis and treatment.

A Diagnosis Branch has been established to manage the growing contract effort in cancer diagnosis. This work is divided into two parts, a program in the automation of cancer cytology, and a program in general cancer diagnosis. At present this consists almost entirely of clinical research on the early diagnosis of lung cancer by sputum cytology, but future expansion to other organ sites and techniques is planned.

LABORATORY OF BIOCHEMISTRY

SUMMARY STATEMENT

ANNUAL REPORT

July 1, 1972 through June 30, 1973

The TUMOR PHYSIOPATHOLOGY SECTION (Dr. P. M. Gullino, Head) was constituted as an independent administrative unit in February 1973. The activities of this unit for the entire fiscal year are included in the separate report of the Laboratory of Pathophysiology.

In the PROTEIN CHEMISTRY SECTION (Dr. E. A. Peterson, Head), an investigation of the factors regulating the maturation of granulocytes has been continued by Dr. W. H. Evans, with the collaboration of Dr. M. G. Mage and current participation of Drs. J. Noseworthy (Research Associate) and F. Huang (Visiting Fellow). Dr. S. R. Himmelhoch, previously a major collaborator, left the Institute during the past year. This work has been aimed at establishing the main metabolic processes and molecular constituents that distinguish normal and mature granulocytes from their precursors and establishing which of the many stages in granulocyte maturation are regulated by humoral or cell-mediated factors, with the ultimate goal of identifying the primary molecular abnormalities in acute and chronic myelogenous leukemia and developing means for reversing or by-passing these defects.

Last year Drs. Evans and Huang reported that mature neutrophilic granulocytes were more active than the immature forms in incorporating radioactive glucosamine into cellular proteins. In subsequent work, they have observed pronounced differences in the glycoprotein patterns obtained when particulate fractions (containing granules and membrane components) of mature and immature neutrophils were extracted with sodium dodecyl sulfate and subjected to gel electrophoresis in the presence of this agent. Their results indicate that a substantial number of new glycoproteins appear in the later stages of neutrophil maturation, whereas the few glycoproteins observed in immature cells are greatly reduced in quantity or disappear.

Using density gradient zonal centrifugation, Drs. Evans and Noseworthy have achieved nearly complete resolution of the primary and secondary granules of guinea pig polymorphonuclear leukocytes (PMNL). The work was aided by their recent development of a new method of exudate induction, which has greatly improved the morphological integrity of the exudate cells and enhanced the purity of the PMNL obtained, and by a refinement of their technique for cell lysis that has provided better recovery of granule enzyme activity with almost complete elimination of contaminating nuclear material. Mrs. M. Wolf is currently extracting and characterizing the proteins of each granule type by gel electrophoresis.

Dr. Evans and Mrs. S. Wilson have developed an assay to be used in projected studies of cell-mediated and humoral regulation in granulocyte maturation and proliferation. Granulocyte precursors are labeled with tritiated thymidine, then placed in a short-term chemically-defined culture system, resulting in a four-fold increase in labeled mature granulocytes in 24-40 hrs. Addition of normal serum or suspensions rich in unlabeled granulocytes inhibit the maturation and proliferation of the labeled immature cells. Medium conditioned by mature granulocytes had no effect. The effects of purified serum fractions will be evaluated in future work.

Dr. Mage, continuing his efforts to develop methods for the specific

isolation of immune cells, has found that although the incubation of normal or immune spleen cells on monolayers of allogenic target cells (either primary macrophage or continuous embryonic fibroblast cultures) removes cytotoxic activity from the nonadherent population, as reported by others, it does not remove graft vs. host activity. Both of these activities have been shown to be due to thymus-derived lymphocytes (T cells), but evidently the T cells that initiate graft vs. host reactions differ with respect to antigen binding from those that mediate cell to cell cytotoxic reactions.

Dr. E. A. Peterson has continued the development of procedures for fractionating proteins by displacement chromatography. The current year has been largely devoted to improving procedures for the synthesis and isolation of the wide range of carboxymethyl dextrans used as displacers and the establishment of ground rules for the control of changes in pH and concentration arising from interaction with the adsorbent. Two widely different procedures for the use of the displacers in protein chromatography have been compared, using human serum globulins and soluble protein fractions from guinea pig liver and spleen as test mixtures. Also, a simple modification has been devised for imposing a smooth pH gradient, using an influent solution of constant composition. The possibility of using these displacers in chromatographic separation of cells and cellular organelles will receive attention in future work.

Last year, Dr. O. W. McBride reported the biological transformation of mouse L cells deficient in hypoxanthine-guanine phosphoribosyl transferase (HGPRTase) by incubation with metaphase chromosomes isolated from the Chinese hamster. The transformed hgprt⁺ cells were detected at a low frequency by growth in a medium that supported only cells that could synthesize this enzyme. Physical characterization of the gene products (HGPRTase) obtained from three colonies showed them to be different from that of wild-type mouse L cell and indistinguishable, by chromatography and electrophoresis, from the donor cell type (Chinese hamster). Dr. McBride has now carried out additional gene transfer experiments confirming these observations. The gene product has been identified as a chromosomal donor species in 10 of 11 independent clones examined and was the product of a revertant in only one. Moreover, no colonies were observed in two gene transfer experiments in which the chromosomes employed had been isolated from mutant cells deficient in HGPRTase. Controls also indicate that the colonies probably could not have arisen through uptake of nuclei rather than chromosomes.

Experiments are underway to characterize the enzyme molecules further by immunological methods and by peptide mapping of tryptic digests. The recent observation that the mutant cell line used in these experiments as the recipient also lacks adenine phosphoribosyltransferase (APRTase) provides an opportunity to examine the efficiency for transfer of two genes (on different chromosomes) in the same experiment. Clones already transformed for hgprt⁺ are therefore being re-examined for the presence of APRTase.

In the BIOSYNTHESIS SECTION (Dr. E. L. Kuff, Head), studies were continued on intracisternal A-particles, virus-like structures that occur occasionally in adult and embryonic mouse tissues but are numerous in many primary and transplanted mouse tumors. Similar particles have now been reported in tumors of rat, guinea pig, hamster and in cultured human tumor cells; they may represent expression of a new type of viral genome analogous to but distinct from those of the C and B-type oncogenic viruses. Collaborators during the past year included Drs. E. Kuff, S. Wilson, D. Marciani (Guest Worker), J. Minna (Laboratory of Molecular Genetics, NHLI), and Mrs. K. Lueders. A-particles isolated

from several mouse tumors were earlier found to contain a distinctive DNA-polymerase activity. With Dr. Wilson, a detailed study of the intracellular distribution of this activity in myeloma cells has been carried out. More than 80% of the homogenate activity was recovered in the cytoplasmic extract and the cytoplasmic activity appeared to be exclusively associated with the A-particles. This finding was confirmed for neuroblastoma cells using a new isolation procedure which yields complete recovery of both A-particle enzyme and antigen. Thus the enzyme activity appears to be a specific biochemical marker for mouse intracisternal A-particle.

Dr. Marciani is continuing studies on the isolated structural proteins of these particles with results that will be summarized in a subsequent report. In collaboration with Dr. Minna, Mrs. Lueders has assayed A-particle antigen levels in a number of hybrid cell lines resulting from fusion of mouse neuroblastoma cells (A-particle rich) with cells either low in particles (e.g., mouse L-cells) or devoid of them (mouse 3T3 cells, rat glia). Expression of the antigen appeared as a dominant trait with levels in the hybrids approximating those of the high-antigen parent. No evidence was obtained for the action of a repressor contributed by the A-particle negative cells.

With Dr. A. Matsukage (Visiting Associate) and Mrs. E. Bohn, Dr. Wilson has systematically fractionated extracts of murine myeloma in order to define the types of DNA synthesizing enzymes present in this high specialized yet neoplastic mammalian tissue. Multiple forms of DNA-polymerase activity were distinguished on the basis of their chromatographic behavior, template specificity, reaction conditions, sedimentation properties and intracellular localization. Three forms of DNA-dependent activity were found that appeared to correspond to previously recognized activities. Three other forms of DNA-polymerase, maximally or exclusively active with polyriboadenylate [poly(rA)] as template rather than DNA, were also observed. One of these probably represents the A-particle-associated enzyme mentioned above; another may be a reverse transcriptase of the leukemia virus type. The molecular relationships between these various myeloma DNA-synthesizing enzymes and their possible functional roles in gene replication and/or repair will be the subject of extended investigation.

Dr. E. Thompson and his coworkers have continued studies on the control of specific gene expression, particularly as regulated by steroid action. One experimental model is the steroid-inducible enzyme tyrosine aminotransferase (TAT) in hepatoma tissue culture (HTC) cells. With Dr. D. Aviv (Guest Worker), a number of HTC variant lines showing either high or low inducibility for TAT have been isolated. Both types of cells possess specific glucocorticoid receptors; however in the non-inducible lines, the steroid-receptor complex apparently fails to concentrate in the nuclei in the usual fashion. Variant analysis of this type can help to define the intracellular steps in steroid action as well as the specific biochemical lesions underlying development of steroid-resistance.

Drs. M. Lippman (Research Associate) and Thompson have studied glucocorticoid receptor levels in human leukemic cells. In acute lymphoblastic leukemia, the level of cellular receptors correlate with the degree of clinical and in vitro response to therapy which included glucocorticoids. The leukemic cells of patients who had become resistant to such therapy showed little or no receptor activity and failed to be inhibited in vitro by glucocorticoids. Biochemical assay of specific corticoid receptor levels may thus be of considerable clinical value in predicting whether a given patient will respond to steroid therapy.

Drs. E. Shelton and W. Bennett (Research Associate) in anticipation of studying by electron microscopy the distribution and turn-over of antigen receptors on cell surface membranes have prepared a highly purified, ferritin-labeled antibody specific for the antigen, horseradish peroxidase (HRP). The ferritin-labeled antibody will enable them to visualize antigen on macrophage and lymphocyte surfaces. Dr. Shelton is perfecting the techniques of freeze-dry, shadow replication of cells which will permit the examination of the entire exposed surface at a resolution ten times that of the scanning electron microscope. At the light microscope level, Drs. Shelton and Bennett have discovered that soluble HRP-antibody complexes serve as markers for "B" lymphocytes. This is a very useful tool for identifying these lymphocytes in mixed cell populations.

Dr. Shelton has also continued the fine structural analysis of various biomacromolecules. In collaboration with Dr. T. Deuel (Dept. of Medicine, Univ. of Chicago) she has observed that rat liver phosphoribosylpyrophosphate synthetase occurs in linear stacks of a monomeric form. The substructure of the molecule and the effect of stacking on enzyme activity are under study. Finally, in collaboration with Dr. L. Steiner (Dept. Biol., MIT), she had identified another tetrameric macroglobulin. This form of antibody, heretofore identified only in certain fishes, was found in the bull frog R. catesbiana.

Within the NUCLEIC ACIDS SECTION, Dr. Schneider (Head) is continuing his studies on cytoplasmic DNA and has developed a new method for separating mitochondrial and microsomal DNA directly from homogenates of liver without actually isolating the mitochondria and microsomes as discrete fractions. This method is based upon a differential solubility of the two types of DNA in detergent solutions and is important not only because it provides a new method that should facilitate wider studies of cytoplasmic DNA, but also because it furnishes badly needed independent evidence that microsomal DNA exists as such in the cytoplasm and is not simply a preparative artefact. Further evidence for this latter conclusion has come from another study made with Dr. Kidwell of this laboratory and Dr. Smith, and Mrs. Neisses-Rouiller of the Laboratory of Biology. This work demonstrated that DNA was associated with the endoplasmic reticulum (microsomes) of luteal cells of the ovary as shown by autoradiographs of these cells obtained from animals after ³H-thymidine administration. These studies also showed that the hormone, prolactin, produced striking changes in the activity of both mitochondrial and microsomal DNA in the luteal cells as shown by grain counts associated with these organelles in autoradiographs.

Dr. Anderson, with Drs. Garvey, Glick and Millar and Mr. Liacouras, has continued to focus her attention on two of the enzymes involved in the synthesis of uridine nucleotides, compounds which are key intermediates in both RNA and DNA synthesis as well as in the formation of glycoproteins and heteropolysaccharides. Uridine kinase, which catalyses the synthesis of UMP from uridine and ATP, has been purified 150-fold from a fluorouridine-sensitive mast cell neoplasm and its properties have been examined. In addition to the natural substrates uridine and cytidine, the enzyme was also able to phosphorylate fluorouridine and aza analogs of cytidine and uridine, but could not phosphorylate deoxyribonucleosides or cytosine arabinoside. The same enzyme has also been purified 20-30 fold from a fluorouridine resistant mast cell tumor subline. The enzyme level in this tumor was only 10-20 percent of that in the sensitive tumor and was characterized by pronounced lability in the partially purified state. Further comparisons between the kinase in the resistant and sensitive tumors are in progress. Uridylate kinase, which catalyses the

synthesis of UDP from UMP and ATP, has also been the subject of further investigation. Kinetic data indicate that this enzyme like the uridine kinase provides its catalytic function by means of a sequential mechanism rather than the ping-pong mechanism previously postulated. This conclusion was reached after the development of a new assay procedure and the extensive use of computers for curve plotting and statistical evaluations.

Dr. Kidwell has continued his work on the replication of DNA in synchronized tissue culture cells. His previous work had shown that the synthesis of satellite DNA occurred during the first two hours of the S-phase with a second period of synthesis during later S-phase. In order to rule out the possibility that bulk nuclear DNA and satellite DNA were made from different precursor pools of TTP, cells were labelled with pulses of different durations. It was found that the proportion of counts in satellite DNA and in bulk DNA was constant and independent of the pulse length, therefore making it unlikely that different pools were involved. This was further verified by kinetic analysis of ³H-thymidine accumulation in DNA which revealed that the size of the TTP pool used for replication was about the same as total cellular TTP pool. These experiments were carried out with Dr. Wittes. Other experiments have been carried out with Dr. Colyer on a new polymer formed from NAD and localized in the nucleus, which has been postulated to block DNA synthesis. It was found that the synthesis of the polymer, poly-ADP-ribose, occurred in two bursts during the S-phase of the cell cycle. Both of these bursts were inhibited by nicotinamide or thymine while DNA synthesis was not inhibited at all during poly-ADP-ribose formation and only 50 percent subsequent thereto. These results suggest that poly-ADP-ribose synthesis rather than its degradation, may be a prerequisite for DNA synthesis.

Dr. Otani has continued his efforts to synthesize β -hydroxy analogs of amino acids for testing as potential anti-tumor agents. He has prepared the four isomers of β -hydroxynorleucine and their chloroacetyl derivatives and confirmed their purity. He has also prepared β -hydroxyhomomethionine and β -hydroxymethoxinine in quantities sufficient for the separation of each of the four isomers of each compound. The selection of these compounds for synthesis and testing is based upon the fact that they are close analogs of the natural amino acids, norleucine and methionine, and also on previous studies which showed that unresolved β -hydroxynorleucine showed inhibitory activity in several microbial testing systems and also against a transplantable tumor. Sufficient amounts of each of the norleucine isomers are now available for testing in microbial screening systems.

In the CYTOCHEMISTRY SECTION, Dr. D. Burk (Head, in collaboration with investigators at the National Bureau of Standards, and the Naval Ordnance Laboratory, initiated a study bearing on the possible use of nuclear magnetic resonance in the diagnosis of cancer. A mouse's tail, with and without a malignant melanoma growing on it, was placed in a suitable wire coil that formed a spectrometer probe, for irradiation and signal detection. Measurements of "T₁", taken as a function of time, revealed a different value in the presence of the tumor, namely, about 0.6 - 0.8 seconds relaxation time compared to 0.3 seconds in the absence of the tumor. Such measurements are said to represent the first taken with a living animal, and generally confirm results recently reported under in vitro conditions on tissues. By using magnets with larger sample space, experiments could be performed on larger animals and even humans, and interested investigators in New York and Berkeley have informed

Dr. Burk that they possess magnets capable of holding humans. If this technique can be developed and applied at a practical level, competent technicians might be able to test a patient in a matter of minutes.

During the past year, the Division of Cancer Treatment, NCI, has tested the activity of amygdalin (laetrile) against a battery of tumors including Ll210, Walker, P388, B16, and Lewis lung. In one experiment with the Lewis lung tumor, treated mice showed some increase in survival time over controls; however, this was not confirmed in a repeat test. There was no suggestion of activity against the other tumors. On the basis of their experience, DCT does not regard amygdalin as an active compound. However, Dr. Burk has monitored these same results and provided a "proper interpretation" of the data based on the Lewis lung testing, namely that "It is no longer possible for NCI to truthfully report that no amygdalin efficiency has ever been found in NCI-conducted studies."

Dr. Mark Woods, a member of the Cytochemistry Section who has been associated with the National Cancer Institute for more than 25 years, retired at the end of this fiscal year. During this past year, in collaboration with Dr. M. G. Vlahakis and Dr. W. E. Heston (Laboratory of Biology, NCI), he extended his investigations of many years duration on the regulation of glucolysis in normal and cancer cells, studying glucolysis in primary hepatomas and mammary tumors in mice of certain high tumor-incidence strains. His summary of progress follows. "From studies in diverse types of normal tissues and different types of tumors, it is clear that essentially the same basic types of steroid-insulin control of glucolysis occurs in all tissues. Where the relevant non-malignant cells of origin have been available for comparison the derived cancer cells have shown not only increased rates of anaerobic glucolysis, but probably more important, decreases in susceptibility to steroid-induced inhibition of glucolysis. There is considerable evidence that glucolytic capacity also plays a crucial role in the functioning of defensive cells of the host reticuloendothelial system. Successful chemotherapy may depend upon knowledgeable exploitation of differences in glucolytic regulation in cancer and in the normal defensive cells."

Serial No. NCI-333
1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The biochemical basis for defective maturation in myelogenous leukemia

Previous Serial No.: Same

Principal Investigator: Warren H. Evans

Other Investigators: Michael G. Mage, Freesia Huang, S. Ralph Himmelhoch, Margaret Wolf, Shirley Wilson, Elbert A. Peterson, and John Noseworthy

Cooperating Units: None

Man Years:

Total: 4-1/2

Professional: 4-1/2

Other: 0

Project Description:

Objectives: 1) To identify the main molecular constituents and metabolic processes which distinguish mature granulocytes from their immature precursors. 2) To determine whether the major structural and biochemical changes associated with granulocyte maturation are regulated by factors present in body fluids and/or by cell-mediated factors. 3) To use the information gained from (1) and (2) to: (a) identify the primary molecular abnormalities associated with the arrested maturation of granulocytes in acute and chronic myelogenous leukemia and possibly develop methods for the early diagnosis of leukemia, (b) develop specific biological and/or biochemical methods for inducing leukemic cells to develop some or all of the functional characteristics of mature granulocytes as a means of partial or complete restoration of host defense mechanisms in leukemia patients.

Methods Employed: Leukocytes are isolated from bone marrow, blood, and inflammatory exudates by a variety of methods. Subcellular fractions are prepared by differential and zonal centrifugation and further fractionated by chromatography and electrophoresis. Immunological reagents, including

fluorescent antibodies, are prepared from the purified fractions and used to follow the development of related material during maturation of the cells. The maturation of enriched cell populations is studied in soft agar and stationary tissue culture.

Major Findings: 1) Analysis of Molecular Constituents in Maturing Granulocytes: In a previous study we showed that, compared to immature neutrophils, mature neutrophils have a greater activity in incorporating radioactive glucosamine into cellular proteins. The increase in sugar incorporation might reflect changes in either the synthesis or turnover of glycoproteins during cell maturation. We have investigated this question further using highly enriched fractions of mature and immature neutrophil granulocytes prepared by methods previously developed in this laboratory. The protein and glycoprotein composition of sodium dodecyl sulfate (SDS) extracts of the postnuclear particulate fractions of these cells were compared by SDS-gel electrophoresis. Pronounced differences were observed in both the protein and glycoprotein electrophoretic patterns obtained with these extracts. There were 12 prominent protein bands in the patterns of mature neutrophils which also stained for glycoproteins. With one exception, these bands were either greatly reduced in intensity or were undetectable at corresponding positions in the patterns of immature cells. The patterns of immature neutrophils revealed 3 prominent glycoprotein bands, 2 of which were absent and the third greatly reduced in intensity at the corresponding positions in mature cell patterns. These results indicate that a number of new glycoproteins appear in the later stages of neutrophil maturation whereas other glycoproteins, present in immature cells, are either absent or greatly reduced in amount.

Work continues on the development of methods for separating the primary and secondary granules of guinea pig polymorphonuclear leukocytes (PMNL). In contrast to the previously reported purification of peroxidase-containing granules from eosinophil-enriched (by Trichinella antigen sensitization) guinea pig peritoneal exudates, both peroxidase-containing (primary) and alkaline phosphatase-containing (secondary) granules are being prepared from guinea pig exudates enriched in mature PMNL and virtually free of eosinophils. We have developed a new method of exudate induction which has greatly improved the morphologic integrity of the exudate cells and has enhanced its mature PMNL purity. Further quantitative refinement of the technique for cell lysis has led to improved recovery of granular enzymatic activity with almost complete elimination of contaminating nuclear material.

Sucrose density gradient zonal centrifugation of these PMNL lysates has allowed nearly complete resolution of the two granules. The more-rapidly-sedimenting primary granules show a unimodal distribution within the dense portion of the gradient, while the slower-sedimenting secondary granules

distribute themselves bimodally in the lighter fractions. At present, the proteins of each granule type are being extracted and examined by gel electrophoresis.

2) Search for Granulopoietic Regulators: A method has been developed for studying the effect of cell-mediated and/or humoral factors on granulocyte maturation and proliferation. Granulocyte precursors are labelled with tritiated thymidine, washed, and then placed in a short-term, chemically-defined culture system previously developed in the laboratory. In 24-48 hours there is a four-fold increase in labelled mature granulocytes in the culture flask. Preliminary studies indicate that the addition of suspensions rich in unlabelled granulocytes to the cultures inhibit the maturation and proliferation of labelled immature granulocytes. Normal serum also produces similar inhibitory effects. Medium conditioned by mature granulocytes appears to have no effect on maturation or proliferation in this system.

Significance to Cancer Research: Understanding of normal and leukemic granulocyte maturation depends upon clarification of the molecular processes whereby the cell controls the appearance and disappearance of specific components. Such information has never been obtained at the biochemical level in a systematic fashion similar to that achieved in morphological studies of normal and leukemic granulocytes. Any biochemical differences in the maturation processes observed between normal and leukemic leukocytes will be exploited for purposes of developing techniques, presently unavailable, for the early diagnosis of leukemia and for improved treatment, especially with regard to the possibility of reversing the arrested maturation and decreased phagocytic activity of leukocytes from myelogenous leukemia.

Proposed Course of Research: 1) Analysis of Molecular Constituents in Maturing Granulocytes: Work is now aimed at determining the subcellular location of glycoproteins in order to gain some knowledge of their function in granulocyte development. Of particular interest will be the examination of the protein and glycoprotein patterns of leukocyte granules which we have been able to isolate from cell homogenates by density centrifugation. We also plan to develop techniques for isolating and characterizing the constituents of cell membranes from mature and immature granulocytes, with special emphasis on changes in macromolecular components on the outer surface of the cell. Information obtained from these studies will be applied to studies on normal and leukemic human granulocytes.

2) Search for Granulopoietic Regulators: The short-term culture system we have developed will be used to continue studies aimed at determining which specific structural and biochemical steps in cell maturation are subject to external control by humoral and/or cell-mediated mechanisms. Since we have

shown that granulocyte maturation and proliferation can occur in chemically-defined McCoy's 5A medium, we plan to determine which of the constituents in this medium are essential for normal cell maturation. We also propose to fractionate normal serum to determine the nature of the nondialyzable factor or factors which retard granulocyte maturation and proliferation in vitro. The effects of these media and serum factors on granulocyte maturation at the biochemical level will be examined by gel-electrophoretic techniques and by analysis for a variety of enzymes which are known to appear during normal granulocyte maturation but which fail to develop in leukocytes from patients with acute and chronic leukemia.

Honors and Awards: None

Publications:

1. Huang, F. L., and Evans, W. H.: Incorporation of D-[6-³H]glucosamine into maturing bone marrow granulocytes. Biochim. Biophys. Acta 273: 157-164, 1972.
2. Grieshaber, C. K., and Evans, W. H.: Effect of serum on amino acid incorporation into protein of guinea pig marrow granulocytes. J. Reticuloendothel. Soc. 12: 485-496, 1972.
3. Wilson, S. M., and Evans, W. H.: Quantitative cytological studies of the maturation of bone marrow neutrophils in short-term suspension cultures. J. Reticuloendothel. Soc. 12: 514-524, 1972.
4. Hymer, W. C., Evans, W. H., Kraicer, J., Mastro, A., Davis, J., and Griswold, E.: Enrichment of cell types from the rat adenohypophysis by sedimentation at unit gravity. Endocrinology 92: 275-287, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-333

Title: The biochemical basis for defective maturation in myelogenous leukemia

Principal Investigator: Warren H. Evans

Section: Protein Chemistry Section

Lab/Branch: Laboratory of Biochemistry

Goal: To identify the primary molecular abnormalities associated with the arrested maturation of granulocytes in acute and chronic myelogenous leukemia and to develop means for reversing these defects.

Approach: Work is first aimed at identifying the main molecular constituents and metabolic processes which distinguish normal mature granulocytes from their immature precursors and establishing which of the many steps involved in granulocyte maturation are regulated by factors present in body fluids and/or by cell mediated factors. The results will be compared with those obtained from similar studies on leukemic cells at corresponding stages of maturity in order to determine the nature and potential reversibility of the arrested maturation steps. Biochemical analyses of molecular constituents are carried out on mature and immature granulocytes isolated from blood and bone marrow by velocity sedimentation and density centrifugation procedures developed in this laboratory. The effects of external cell regulators on the synthesis of specific components are studied in a chemically defined culture system previously developed in this laboratory.

Progress: Our studies indicate that a number of new proteins, chiefly of the glycoprotein type, appear in the later stages of granulocyte maturation, whereas other glycoproteins, present in immature cells are absent or greatly reduced in amount in mature cells. These changes are presumably related to the development of specific cell functions that also appear in later stages of cell maturation. We have also developed a method for studying cell-mediated regulatory mechanisms of granulocyte maturation in tissue culture.

Significance for Cancer Research (NCP Objective 2 Approach 3) The main thrust of this work is to develop biochemical methods for the early diagnosis of granulocytic leukemia and methods for inducing leukemia cells to develop some or all of their functional properties as a means of partially or completely restoring host defense mechanisms in leukemia patients.

Level of Effort: Professional Man-Years: 3

Serial No. NCI-5203

1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Development of immunochemical and physical methods for the purification and characterization of immunocytes and their subcellular components

Previous Serial No.: Same

Principal Investigator: Michael G. Mage

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1-1/2

Professional: 1-1/2

Other: 0

Project Description:

Objectives: The goal of this project is the development of methods for the specific isolation of immune cells, particularly for antigen-reactive cells involved in cellular immune reactions, and for their subcellular fractionation in order to study the mechanisms involved in the development of immune reactivities and immune macromolecules.

Methods Employed: To obtain antigen-reactive lymphocytes, inbred mice are immunized with allogeneic tumor cells. Immune spleen cell suspensions are incubated on allogeneic and syngeneic target cells, and the cells that do not adhere to the target cells are tested for depletion of cytotoxic and graft-versus host (GVH) activity. Cytotoxic activity is measured by the release of ^{51}Cr from target cells, and GVH activity is measured by the Simonsen spleen weight assay in neonatal mice.

Major Findings: Incubation of normal or immune spleen cells on monolayers of allogeneic target cells (either primary macrophage or continuous embryonic fibroblast cultures), a procedure shown by Brondz and others to remove up to 4/5 of the cytotoxic activity from the nonadherent population, does not

deplete GVH activity. Although both these activities have been shown to be due to thymus-derived lymphocytes (T cells), these results indicate that the T cells that initiate GVH reactions differ with respect to antigen-binding from those that mediate cell to cell cytotoxic reactions, and that the procedures used to remove cytotoxic activity from immune spleen cell populations do not remove GVH activity. This difference in antigen binding may be due to lack of the antigens needed for the GVH reaction on fibroblasts and macrophages, or to different conditions of incubation needed for GVH antigen binding.

Significance to Cancer Research: These studies on the specific separation of antigen-reactive cells relate to the mechanisms of cell-mediated tumor rejection. Knowledge of the antigen-binding characteristics of immune cells may help in development of specific purifications of immune lymphocytes with anti-tumor reactivities, and may aid in understanding the variation in tumor susceptibility of human beings and make possible control of immune mechanisms of tumor surveillance and rejection.

The GVH reaction is an iatrogenic complication of tumor therapy regimens that include bone-marrow transplantation. A specific separation of GVH-antigen reactive cells might aid in avoiding this complication.

Proposed Course of Research: As an aid to the development of general methods for the specific isolation of antigen-reactive lymphocytes, we plan to further explore the antigen-binding characteristics of immune T-cells, particularly with respect to target-cell antigen specificity and to conditions that favor GVH antigen-binding, utilizing antigen-coated foam columns at 37° in addition to monolayers in tissue culture.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5203

Title: Development of immunochemical and physical methods for the purification and characterization of immunocytes and their sub-cellular components

Principal Investigator: Michael G. Mage

Section: Protein Chemistry Section

Lab/Branch: Laboratory of Biochemistry

Goal: The development of methods for the specific isolation of immune (antigen-reactive) cells and for their subcellular fractionation, in order to study development of and control of immune reactivities.

Approach: Antigen-reactive lymphocytes are incubated with various classes of target cells under a variety of conditions in order to learn how to effect specific separations of antigen-reactive lymphocytes involved in immune destruction of allogeneic normal and neoplastic cells.

Progress: We have found that we can distinguish the antigen-binding characteristics of cytotoxic effector cells from graft-versus-host antigen-reactive cells in that the former bind to allogeneic fibroblasts and macrophages under conditions where the latter do not bind.

Significance for Cancer Research (NCP Objective 2 Approach 1)
Specific purifications of lymphocytes that react with allogeneic cells and with tumor cells may help in developing immunotherapies of neoplasms, and in avoiding graft-versus-host reactions.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-375

1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Homogeneity and structure of proteins

Previous Serial No.: Same

Principal Investigator: Elbert A. Peterson

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 2

Professional: 1

Other: 1

Project Description:

Objectives: To develop methods for the separation and analysis of proteins that will overcome present limitations and to apply these methods to the purification of specific proteins for the study of their function and structure.

Methods Employed: A variety of polyanions and polycations differing in molecular size, charge density, and chemical composition are prepared by appropriate reactions. Most of these are ionic derivatives of low molecular weight dextrans but other types are being explored. They are evaluated as displacing agents, using test protein mixtures, on cellulose ion exchangers. Effluent fractions are examined by electrophoresis in slabs of polyacrylamide gel and by appropriate assay.

Major Findings: Improvements have been made in the synthesis and isolation of the carboxymethyl dextrans (CM-dextrans) employed as displacers in this work. The use of LiOH instead of NaOH in the reaction has permitted the direct isolation of the free acid or salt forms of the CM-dextrans as dry solids by precipitation with alcohol and acetone. This method replaces the time-consuming dialysis and evaporation procedures initially employed, which were, moreover, unsuitable for isolating the lower molecular weight displacers (5000-10,000 MW) that have been investigated in this period. Also, substantial

increases in the level of substitution have been achieved by reduction of the water content of the reaction mixture to the minimum required for mixing.

Two basic procedures (and several modifications) for the use of these displacers have been compared, using human serum globulins and soluble proteins from guinea pig liver and spleen as test mixtures. In the first method, the DEAE-cellulose column is initially equilibrated with buffer, then the protein sample is applied, followed by a single solution containing a mixture of CM-dextran (in their salt form) having a wide range of affinities, including a quantity of the highest affinity displacer sufficient to saturate the column. In the second method, the column is packed with adsorbent that has been brought from its free-base form to the pH selected for the experiment by the addition of a mixture of CM-dextran as free acids. The protein sample is applied to the column and followed by a solution of displacer having a higher affinity than any of those previously added.

Although the second method appears to be generally better in that it avoids problems arising from the displacement of buffer salt, each method has technical advantages and disadvantages, chiefly in regard to the control of changes in pH and concentration that are caused by interaction with the adsorbent. Progress has been made in working out the ground rules for the suppression of such changes. Also, a very simple modification has been devised for imposing a smooth pH gradient in the second method, using a single influent solution of constant composition. This increases the effectiveness of the displacers in removing protein having very high affinity.

Significance to Cancer Research: Displacement chromatography of proteins, nucleic acids, etc. promises to be of considerable value at any scale of operation and therefore has significance to all research that involves the isolation of such substances. Its immediate significance to the program of this Section, however, lies in its potential application to the fractionation of the small quantities of protein available in each fraction obtained when bone marrow cells are separated into populations of different cell types. The displacers, unlike salt, do not interfere with electrophoresis, and protein fractions obtained by displacement can be directly subjected to electrophoresis or rechromatography without dialysis or concentration. Thus the examination of complex mixtures by a combination of chromatography and electrophoresis on a semi-micro scale is greatly expedited. The simplicity of operation also facilitates automation, so that the two-dimensional analysis of large numbers of fractions becomes feasible.

Moreover, since migration is induced by displacers under conditions of salt and pH in which the migrating molecules are otherwise strongly adsorbed, the possibility arises of chromatographing substances that are

"irreversibly" absorbed in conventional procedures. Moreover, theoretical considerations strongly suggest that many of the enzymes that become labile when purified can be protected from inactivation or loss by substances such as the CM-dextrans if the metal-sequestering properties of the system are taken into account.

Proposed Course of Research: The method, as it develops, will be applied to the subfractionation of t-RNA and to the examination of proteins and nucleic acids in guinea pig bone marrow cells. Its possible application in the separation of cells and subcellular organelles will be explored, using appropriate adsorbent matrices. Also, a stabilizing effect of the CM-dextrans will be looked for using enzymes that have previously been difficult to purify because of their high lability.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-375

Title: Homogeneity and Structure of Proteins

Principal Investigator: Elbert A. Peterson

Section: Protein Chemistry Section

Lab/Branch: Laboratory of Biochemistry

Goal: To develop methods for the separation and analysis of proteins that will overcome present limitations and to apply these methods to the purification of specific proteins for the study of their function and structure.

Approach: Biological mixtures of proteins are separated by displacement chromatography on suitable ion exchangers, instead of by elution with salt. A variety of polyanions and polycations differing widely in molecular size, charge density, and chemical composition are prepared by appropriate reactions. Most are ionic derivatives of low molecular weight dextrans. They are evaluated as displacing agents, using test protein mixtures, on columns of DEAE-cellulose and other ion exchangers. As they move down the column, the displacers and the proteins arrange themselves in the order of increasing affinity and the resulting displacement train is moved off the column by the displacer having the highest affinity, which is provided in excess of the amount required to saturate the column. Effluent fractions are examined by electrophoresis in slabs of polyacrylamide and by appropriate assay.

Progress: Improvements have been made in the synthesis and isolation of the carboxymethyldextrans used as displacers, resulting in substantial increases in the level of substitution and facilitating recovery of the products as dry solids. Two basic procedures (and several modifications) for the use of these displacers have been compared, using human serum globulins and soluble proteins from guinea pig liver and spleen as test mixtures. Progress has been made in establishing the ground rules for the suppression of unwanted changes in pH and concentration that arise from interaction with the adsorbent.

Significance for Cancer Research(NCP Objective 2 Approach 3) Although this work can have significance to all research that involves the isolation of proteins and nucleic acids, its immediate significance lies in the advantages displacement chromatography affords the examination of the proteins of bone marrow cells at different stages of maturation.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-5202

1. Laboratory of Biochemistry
2. Protein Chemistry Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Isolation, fractionation, and characterization of native nucleoproteins

Previous Serial No.: Same

Principal Investigator: O. Wesley McBride

Other Investigators: Michael G. Mage, Elbert A. Peterson

Cooperating Units: None

Man Years:

Total: 2

Professional: 1

Other: 1

Project Description:

Objectives: The determination of the role of nucleic acid-bound proteins in the regulation of the transcription and translation of genetic information, and the biological transformation of cells with isolated and fractionated mammalian metaphase chromosomes as a means of genetic mapping.

Methods Employed: Tissue culture procedures involving propagation of mammalian cells in monolayer and suspension cultures, plating in selective media, cloning, labelling with radioisotopes, selection of cell mutants, and metaphase arrest for chromosome isolation. Metaphase chromosome isolation and purification by isopycnic and velocity sedimentation (including unit gravity procedures), and evaluation of chromosomal DNA molecular weight by zonal density gradient sedimentation. Gene transfer to mammalian cells via uptake of purified metaphase chromosomes and isolation of biologically transformed colonies in selective media. Isolation, quantitation, and characterization of gene products by chromatography, electrophoresis, isoelectric focusing, and immunochemical procedures.

Major Findings: The uptake of metaphase chromosomes by mammalian cells and subsequent replication and expression of chromosomal genes has been

demonstrated. Our previous findings have been extended by additional gene transfer experiments confirming this observation, including biochemical characterization of the gene product from more of the isolated clones. This product hypoxanthine-guanine phosphoribosyl transferase (HGPRTase), has been identified now as chromosomal donor species in 10 of the 11 independent clones examined, whereas it represents the product of a revertant in only one instance. Furthermore, no colonies were observed in two gene transfer experiments where the chromosomes employed had been isolated from mutant cells defective in HGPRTase. Controls also indicate that the colonies probably could not have arisen through uptake of nuclei rather than chromosomes. Experiments utilizing chromosomes isolated by three different methods have all resulted in gene transfer.

Efforts have been made to improve the efficiency of this transformation by several methods including: 1) alteration of the plasma membrane of recipient cells by brief trypsin treatment; 2) comparing transfer in the presence and absence of serum and polycations in the medium; 3) attempts to incorporate chromosomes into liposomes, thereby facilitating fusion with cells; and 4) Binding of surface-active agents (lysolecithin) to the donor chromosomes to facilitate their entry into cells by a non-phagosomal pathway. No striking improvement in the efficiency of transfer has been found by any of these procedures.

Proposed Course of Research: The HGPRTase product from all independently derived clones will be further characterized to provide additional evidence that the appearance of enzyme could not have resulted from a back-mutation of the recipient cells. Collaborative studies with Dr. M. Mage are in progress to identify the species of origin of the enzyme by immunochemical procedures employing species-specific antibody to the HGPRTase product. Purification of the HGPRTase from both parental species and several clones will also permit identification of the species of origin by peptide mapping of tryptic digests.

Chromosomes from diploid human cells (mitogen-stimulated lymphocytes) will be employed in gene transfer experiments to provide further evidence for the general applicability of this procedure and also to permit analysis of the mode of gene transfer, i. e., intact chromosome or free or integrated chromosome fragments. This information will be obtained by analysis of the transformants for other X-linked marker gene products. Reciprocal experiments employing human HGPRT-deficient cells (from patients with Lesch-Nyhan disease) and hamster and mouse donor chromosomes will also be performed.

The recent observation that L_{A9} cells lack adenine phosphoribosyl

transferase (APRTase) provides an opportunity to examine the efficiency for transfer of two genes (located on different chromosomes) in the same experiments under virtually identical conditions and to determine the frequency of transfer of both genes to the same recipient cell. Furthermore, isolated clones transformed for hgp_rt are being re-examined for the presence of APRTase. Failure to observe the presence of both HGPRTase and APRTase in any of these clones would perhaps provide the most convincing demonstration that gene transfer did not occur through uptake of intact nuclei and/or cells rather than chromosomes.

The transfer of chromosomes isolated from cells transformed with SV40 virus to a monkey kidney cell line (CV-1) permissive for SV40 virus replication will be investigated in collaboration with Dr. Robert Martin. This can provide a means for investigating the sites of integration of oncogenic viruses when it is subsequently combined with the use of fractionated metaphase chromosomes.

Methods to increase the efficiency of genetic transfer from metaphase chromosomes will be explored further. Efforts will be made to isolate cell mutants deficient in lysosomal DNase and also to prepare antibody to this enzyme to test the hypothesis that gene transfer is currently limited primarily by lysosomal degradation of chromosomal DNA following uptake.

Significance to Cancer Research: Metaphase chromosome isolation and fractionation provides the most promising approach for the fractionation of mammalian genes and can provide material for the investigation of control elements associated with specific sets of genes. The biological expression of these chromosomes introduced into recipient cells provides a method for mapping of mammalian genes, investigating differences between genetic elements in normal and neoplastic cells, and looking for sites of integration of oncogenic viruses into the mammalian genome.

Honors and Awards: None

Publications:

1. McBride, O. W., and Ozer, H. L.: Transfer of genetic information by purified metaphase chromosomes. Proc. Nat. Acad. Sci. USA 70: 1258-1262, 1973.
2. McBride, O. W., and Ozer, H. L.: Gene transfer with purified mammalian chromosomes. In Silverstri, L. G. (ed.): Possible Episomes in Eukaryotes Lepetit Colloquia on Biology and Medicine. Amsterdam, Netherlands, North-Holland, 1973, Vol. 4. In press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5202

Title: Isolation, fractionation, and characterization of native nucleoproteins

Principal Investigator: O. Wesley McBride

Section: Protein Chemistry Section

Lab/Branch: Laboratory of Biochemistry

Goal: To biologically transform cells with isolated, fractionated, mammalian, metaphase chromosomes as a means of genetic mapping and of determining sites of integration of oncogenic viruses.

Approach: Mammalian chromosomes are isolated from metaphase arrested, ^3H -TdR labelled, mammalian cells by several methods. The chromosomes are purified and fractionated by isopycnic and velocity sedimentation (including unit gravity procedures). Chromosomes isolated from hgp prt^+ (wild-type) Chinese hamster cells are incubated with mouse cell mutant (LA $_9$) deficient in HGPRTase. The cells are subsequently plated in selective media, resulting in the death of unaltered parental A $_9$ cells. Colonies of HGPRT $^+$ cells are cloned, subcultured, and characterized karyologically, by their growth in selective media, and according to stability of phenotype. The gene product is isolated and the species of origin (i. e., chromosomal donor or recipient) is identified by physical and immunological characterization, so that back mutation of parental cells can be excluded.

Progress: The uptake of metaphase chromosomes and subsequent replication and expression of chromosomal genes has been demonstrated. The HGPRTase gene product has been identified as chromosomal donor species by DEAE-cellulose chromatography and gel electrophoresis. The colonies differed from each other in stability of expression of the transferred gene. Efforts to improve the efficiency of gene transfer have not been successful.

Significance for Cancer Research (NCP Objective 2 Approach 3)

Metaphase chromosome isolation and fractionation provides the most promising approach for the fractionation of mammalian genes and can provide material for the investigation of control elements associated with specific sets of genes. The biological expression of these chromosomes introduced into recipient cells provides a method for mapping of mammalian genes, investigating differences between genetic elements in normal and neoplastic cells, and looking for sites of integration of oncogenic viruses into the mammalian genome.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-366
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biosynthesis and assembly of intracellular structural components

Previous Serial No.: Same

Principal Investigator: Edward L. Kuff

Other Investigators: Kira K. Lueders, Dante Marciani, Samuel H. Wilson, John D. Minna and Sarah Stewart

Cooperating Units: Laboratory of Biochemical Genetics, NHLI; Department of Pathology, Georgetown University School of Medicine

Man Years:

Total: 4

Professional: 3

Other: 1

Project Description:

Objectives: To study the synthesis and transport of individual macromolecules and their assembly into complex intracellular components; to study the process of neoplastic transformation in terms of these coordinated functions.

Methods Employed: Cell fractionation by differential and density gradient centrifugation, isolation and gradient fractionation of nucleic acids and nucleoproteins, electron microscopy of isolated nucleoproteins, in vivo and in vitro incorporation of isotopically-labelled precursors into protein and nucleic acid, chromatographic, immunochemical and electrophoretic characterization of specific proteins, tissue culture of normal and transformed cells.

Results: Studies during the past year were concerned primarily with intracisternal A-particles.

1. Isolated A-particles had previously been shown to contain an enzyme activity which specifically promoted the synthesis of poly(dT) on oligo(dT)-

primed natural or synthetic RNA's containing poly(rA) sequences (see previous Annual Report). The subcellular distribution of this activity has now been studied in detail in homogenates of mouse myeloma and neuroblastoma cells. In both cell types the distribution precisely paralleled that of the group specific A-particle antigen. In addition the enzyme activity was not detected in cells lacking A-particles as judged by electron microscopy. The enzyme activity thus appears to be a specific biochemical marker for this type of particle in mouse cells. Aspects of this study are also described in Annual Report NCI-5214, prepared by Dr. S. H. Wilson.

2. Intracisternal A-particles from a number of mouse tumors all contain a major structural protein with molecular weight of 73,000 daltons, and varying lesser amounts of proteins in the size range of 45,000 and 30,000 daltons. Dr. Marciani has isolated these different-sized components by gel chromatography in sodium dodecylsulfate and found that they contain a common antigenic site (or sites). Since the particle proteins appear to be lacking in carbohydrates, it is likely that the common reactivity reflects a shared amino sequence. In this case, the lower molecular weight proteins could arise from specific cleavage of the 73,000 dalton molecule.

3. During the subcellular distribution studies, it was found that the original A-particle isolation procedure, while yielding particles of high purity, gave incomplete recoveries (about 15%) of both total antigen and particle-specific DNA polymerase activity in the final preparation. A simplified new procedure has now been developed which gives essentially complete recovery of these components at some sacrifice in purity. Using this procedure, Mrs. K. Lueders has begun to study the rates of synthesis and turnover of the major structural proteins of the particles and associated membranes in cultured neuroblastoma cells.

4. With Dr. J. Minna (NHLI), A-particle antigen has been assayed in a number of hybrid cell lines derived from parent cells that differ widely in antigen content or in which one parent is devoid of detectable antigen. As described last year, high antigen content was observed in hybrids between neuroblastoma (high) L-cell (low antigen) lines. We have now observed the persistence of high antigen levels in crosses between neuroblastoma and A-particle negative mouse (3T3 fibroblasts) or rat (glia) lines. Thus, expression of this protein appears as a dominant trait in the hybrid cells; the study has provided no evidence for operation of repressor contributed by the antigen-negative parent cell.

5. In collaboration with Dr. Sarah Stewart (Georgetown University) we have recently prepared fractions from cultured human tumor cells (Gomez line) in which intracisternal A-type particles have been induced by treatment with

iododeoxyuridine and dimethylsulfoxide. These preparations have not reacted in complement fixation with antiserum against mouse A-particles.

Significance to Cancer Research: Intracisternal A-particles may represent the expression of an integrated viral genome which is distinct from the recognized oncogenic RNA viruses but possibly as widespread in normal and neoplastic mouse cells. During the past year, additional interest has attached to this type of particle because of the specificity of association of the DNA polymerase activity, a report that the isolated mouse particles contain some high molecular weight RNA, and further descriptions of intracisternal A-particle induction in transformed rat and human cells. It is important to understand the regulation of A-particle expression and determine whether this genome is biologically significant in neoplastic transformation.

Proposed Course of Research: The possible intracellular function of A-particle associated DNA polymerase will be examined. The enzyme will be further investigated to determine whether it represents a unique molecular species or a more general cellular polymerase which acquires unique enzymatic properties in consequence of binding to the particles will be further characterized; the possibility that identical or related proteins are more generally distributed in the membranes of transformed cells will be examined. Synthesis and assembly of the A-particles will be quantitated in cultured neuroblastoma cells, especially in relationship to rate of cell multiplication and cell cycle stage.

Honors and Awards: None

Publications: (See also Project Report NCI-5214)

1. Wivel, N. A., Lueders, K. K., and Kuff, E. L.: Structural organization of murine intracisternal A-particles. J. Virol. 11: 329-334, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-366

Title: Biosynthesis and assembly of intracellular structural components

Principal Investigator: Edward L. Kuff

Section: Biosynthesis Section

Lab/Branch: Laboratory of Biochemistry

Goal: To define critical phenotypic changes in neoplastically transformed cells and determine the cause of these changes.

Approach: Currently, to characterize biologically and biochemically a type of virus-like particle (intracisternal A-type particle) that is associated with many mouse tumor cells and has also been seen in transformed cells of rat, guinea pig, cat, and human origin; to determine whether these particles reflect the expression of an integrated viral genome that is biologically significant in neoplastic transformation. Techniques employed include a range of cell fractionation and viral isolation procedures, biochemical assays of molecular and enzymatic properties, tissue culture procedures, and study of hybrid lines resulting from somatic cell fusion.

Progress: Intracisternal A-particles have been purified from several mouse tumors and shown to contain structural proteins, group specific antigen and DNA polymerase activity different from those associated with the B- and C-type oncogenic RNA viruses. The particles may contain a high molecular weight RNA. The characteristic antigen is associated with structural proteins of different sizes and the possibility is raised that the lower molecular weight components may be more generally distributed in the membranes of transformed cells. Expression of A-particle antigen is a dominant trait in hybrid cells resulting from fusion of A-particle-rich cells with cells devoid of these particles.

Significance for Cancer Research (NCP Objective 3 Approach 2) These particles may be the morphological indication of a previously unrecognized viral genome that may play a role in the formation of some tumors.

Level of Effort: Professional Man-Years: 3

Serial No. NCI-5214

1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Gene replication and repair in mammalian cells

Previous Serial No.: Same

Principal Investigator: Samuel H. Wilson

Other Investigators: Akio Matsukage, E. L. Kuff, J. V. Miller, Jr.,
E. B. Thompson, B. K. Shrier, and K. Stromberg

Cooperating Units:

Man Years:

Total: 2-3/4

Professional: 1-3/4

Other: 1

Project Description:

Objectives: The broad objective of this research program is to understand mechanism and regulation of DNA synthesis in eucaryotic cells. During the current year the thrust of our research has been toward gaining a better understanding of the types of enzymatic machinery available for DNA synthesis in a highly specialized yet rapidly growing neoplastic mammalian tissue, murine myeloma MOPC-104E.

Methods Employed: In order to investigate the DNA synthesizing enzymes in murine myeloma MOPC-104E, a procedure was developed for the extraction and systematic fractionation of the maximum number of DNA polymerase activities. The procedure was based on a combination of subcellular fractionation, two-step ion-exchange column chromatography and assays for DNA polymerase activities using three different types of reaction conditions. Cell fractionation procedures and measurements of reaction products were performed using conventional techniques of density equilibrium centrifugation, rate-zonal centrifugation, scintillation spectrophotometry and various types of column chromatography.

Major Findings:

1) A Type of DNA Polymerase Activity Present in Myeloma MOPC-104E Extracts Co-Sedimented with Intracisternal A-Particles and Appears to be Tightly Associated with the A-Particle Core. Previous results indicated that a DNA polymerase activity was present in preparations of intracisternal A-type particles isolated from several murine tumors. In the current study, this enzyme activity was not released from A-particles after treatment with 1 M KCl, 1% deoxycholate, 1% Tween 80 or 1% Triton N-101. Similar procedures have been shown to remove a portion of the outer membrane shell of A-particles, thus releasing the A-particle core.

The DNA polymerase activity in various myeloma MOPC-104E extracts possessed isopycnic sedimentation properties that were virtually identical to those of an antigen in the main A-particle structural protein.

Thus, no evidence was obtained for the association of the DNA polymerase activity with material other than A-particles, and the enzyme was not released by procedures that have been shown to wash the A-particle core free of most of its outer membrane shell.

2) Appreciable Levels of the Type of DNA Polymerase Activity Associated with A-Particles was not Found in Murine Tissues Devoid of A-Particles. Extracts from several murine tissues that were either devoid of or contained very low numbers of A-particles possessed no detectable DNA polymerase activity. No inhibitors of enzyme activity were found when a previously characterized sample of A-particle was mixed with these extracts and assayed again. Similarly, prepared extracts from cells containing numerous A-particles all possessed DNA polymerase activity. Thus, the presence of the DNA polymerase activity was related to the presence of A-particles in tissues.

3) Studies on the Subcellular Distribution of the Type of DNA Polymerase Activity that is Associated with Intracisternal A-Type Particles Showed that the Enzyme Activity and A-Particles are Co-Purified During the A-Particle Isolation Procedure. Approximately 80% of this type of enzyme activity in myeloma MOPC-104E fractionated as a particulate cytoplasmic constituent that was not solubilized by treatment with 1.7% Triton X-100 and 135 mM potassium citrate. Very little activity was detected in the soluble fractions isolated during the subcellular distribution experiments. The specific activity of the enzyme in preparations of purified A-particles ranged from 30 to 60 fold higher than the specific activity of the crude homogenate, depending on the type of myeloma tumor used in the studies.

4) DNA Polymerase in Murine Myeloma was Systematically Fractionated; Six Distinct Major Forms of Enzyme were Detected. Systematic fractionation of myeloma MOPC-104E extracts revealed six structurally distinct forms of DNA polymerase. These forms of enzyme were characterized on the basis of their ion-exchange column chromatography, glycerol gradient sedimentation, sulfhydryl properties and subcellular locations.

The results indicated that myeloma like many other mammalian tissues contains a high molecular weight (6.1S) cytoplasmic DNA polymerase and a low molecular weight (2.5S) DNA polymerase that was not inhibited by sulfhydryl blocking agents. A second form of low molecular weight (2.2S) DNA polymerase that was located in the cytoplasmic membrane fraction and the nuclear fractions were also resistant to sulfhydryl blocking agents. A second form of high molecular weight (5.9S) DNA polymerase was also found. This enzyme appeared to correspond to an enzyme previously detected in HeLa cell nuclei. Two additional previously unrecognized forms of DNA polymerase were detected. One of these, a 6.3S form of activity may correspond to the A-particle associated DNA polymerase while a 4S form of activity may be similar to the C-type particle DNA polymerase. A minor form of DNA polymerase that sedimented at 9.3S was located in the cytoplasmic membrane fraction and may correspond to the mitochondrial DNA polymerase. Some fractions from ion-exchange column chromatograms contained high levels of terminal transferase enzyme activity, and also a deoxynucleotide incorporation activity that was not the result of DNA synthesis.

5) Purification and Characterization of a Low Molecular Weight Murine Myeloma DNA Polymerase. The isolation of a low molecular weight form (2.2S) of DNA polymerase was described above in Section 4. This enzyme was located primarily in the cytoplasmic membrane fraction. The enzyme isolated by DEAE-cellulose and phosphocellulose column chromatography, was further purified by ion-exchange column chromatography on hydroxyapatite and then by affinity column chromatography on DNA-cellulose. The reaction kinetics and physical properties of this highly purified enzyme were investigated. The enzyme required a template and primer for activity, preferred synthetic DNA and RNA templates, was not inhibited by the sulfhydryl blocking agents, N-ethylmaleimide or iodoacetamide, was inhibited by high salt and was two times more active in the presence of the optimal concentration of manganese (0.25 mM) than the optimal concentration of magnesium (4.6 mM).

6) Poly(dT) Inhibition of Cell-Free Protein Synthesis Appears to be the Result of a Block in Peptide Chain Initiation. The mechanism of inhibition of globin synthesis in a Krebs II ascites cell system was further investigated. Poly(dT) had little effect on the initial rate of peptide chain elongation by pre-existing polyribosomes, but was a potent inhibitor of protein synthesis directed by

exogenous messenger RNA. The principal difference between these two systems is that in the case of polyribosomes peptide chain initiation does not have to occur in order for protein synthesis to occur. Therefore, it appeared that poly(dT) inhibited peptide chain initiation. These studies are described in detail in Dr. Thompson's report (Serial No. NCI-5210).

7) Optimal Methods for Determining the Amount of Radioactivity in Samples of DNA were Investigated. Studies on methods for collecting and counting of ^3H DNA indicated that the highest counting efficiencies were obtained using glass fiber filters, NCS for solubilization of DNA and a toluene:Liquifluor scintillation mixture. However, glass fiber filters were not effective in collecting single stranded DNA of 185,000 molecular weight. Quantitative recovery of both this low molecular weight DNA and high molecular DNA was obtained with nitrocellulose filters. The optimal counting procedure using nitrocellulose filters involved solubilization of both DNA and filter with cellosolve and counting in Aquasol scintillation fluid. Application of both of these methods of collecting and counting ^3H DNA to assays for DNA polymerase activity was investigated.

8) Selective Release and Partial Purification of the DNA Polymerase Associated with Avian Myeloblastosis Virus. A rapid single-step procedure was developed in collaboration with Dr. K. Stromberg, Laboratory of Biology, NCI, for the solubilization and partial purification of the AMV DNA polymerase. The core component was liberated from intact virus and was then exposed to high salt concentration by sedimentation through a discontinuous sucrose gradient containing 1 M NaCl. DNA polymerase activity was recovered in the gradient and was 10-fold purified relative to the starting intact AMV. This procedure is an important improvement over solubilization of DNA polymerase from intact virus without prior core isolation because of the removal of non-viral contaminants and membrane components before solubilization of the enzyme.

Significance to Cancer Research: A detailed understanding of the mechanism and regulation of DNA synthesis is one of the most important areas of research in the study of the molecular biology of cancer. Many approaches are being used to understand DNA synthesis in normal and neoplastic cells and advances in our understanding on all levels will prove useful in preventing, treating and ultimately controlling cancer. Regulation of DNA synthesizing enzymes is necessary for the maintenance of a rapid rate of cell division and the action of DNA synthesizing enzymes may be necessary in some cases for the development of the transform state.

Proposed Course of Research:

1) To determine the DNA polymerase content of murine tissues in various

stages of growth regulations using the methods of systematic fractionation and detection of DNA polymerases described above. Cells in the process of active replicative DNA synthesis and cells that are inactive in DNA synthesis will be studied as well as cells in the process of repairing of damaged DNA.

2) To extract, purify and characterize the A-particle associated DNA polymerase.

3) To further purify the forms of murine myeloma DNA polymerases that have been isolated and determine their biochemical, immunological and physical properties.

Honors and Awards: None

Publications:

1. Wilson, S. H., Kuff, E. L., Bohn, E. W., and Lueders, K. K.: Studies on DNA synthesis in murine myeloma: II. Activation of latent RNA-dependent DNA polymerase activity in membrane fractions. Biochem. Biophys. Res. Commun. 49: 1093-1099, 1972.
2. Wilson, S. H., Kuff, E. L., Bohn, E. W., Lueders, K. K., and Matsukage, A.: DNA polymerase in association with intracisternal A-type particles. In Wells, R. D. and Inman, R. (Ed.): DNA Synthesis. Baltimore, Md., University Park Press, 1973, In press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5214

Title: Gene replication and repair in mammalian cells

Principal Investigator: Samuel H. Wilson

Section: Biosynthesis Section

Lab/Branch: Laboratory of Biochemistry

Goal: To understand the mechanisms of regulation of DNA synthesis in normal and neoplastic tissues.

Approach: To investigate the enzymes that are capable of promoting DNA synthesis in a highly specialized yet rapidly growing neoplastic tissue, murine myeloma. Cell-free extracts are prepared and tested for DNA polymerase activity. DNA polymerase activities detected are purified using ion-exchange and affinity column chromatography and differential centrifugation. After partial purification the enzyme activities are characterized with regard to physical and biochemical properties.

Progress: 1) Systematic fractionation of extracts from myeloma revealed that this tissue contains six major forms of DNA polymerase. 2) A DNA polymerase activity detected in preparations of intracisternal A-type particles is firmly associated with the particle. The enzyme activity cofractionates with the A-particle during its purification.

Significance for Cancer Research (NCP Objective 3 Approach 2)
Advances in our understanding of DNA synthesis will be useful in preventing, treating and ultimately controlling cancer.

Level of Effort: Professional Man-Years: 1-3/4

Serial No. NCI-5217

1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

NIH-PHS
Individual Project Reports
July 1, 1972 through June 30, 1973

Project Title: Study of mechanisms of information transfer between cells of nervous system origin

Previous Serial No.: Same

Principal Investigator: Samuel H. Wilson

Other Investigators: R. Pertel

Cooperating Units: Laboratory of Biochemical Genetics, National Heart and Lung Institute

Man Years:

Total: 1/8

Professional: 1/8

Other:

Project Description:

Objectives: To develop techniques and procedures for investigation of communication between cells in the nervous system.

Methods Employed: Layer culture of neuroblastoma cells and the free-living nematode, *C. elegans*, was performed in plastic petri dishes in conventional culture media. A new assay based on the principle of isotope dilution was developed and used to determine the amount of histamine in tissues.

Major Findings:

1) Murine Neuroblastoma C-1300 Cells in Culture Contain Histamine. The histamine content of uncloned neuroblastoma C-1300 cells in the stationary phase of growth was found to be 187 ng/g cells. This level of histamine is within the range found in mammalian brain. The assay for histamine which was based on a isotope dilution technique was not influenced by low recovery of histamine during extraction of inhibitors of the histamine fluorescence assay that were present in cell extractions. Both of these latter complications were encountered when a conventional histamine assay was used.

2) The Free-Living Nematode, C. elegans, Contains a High Level of Histamine. 350 ng histamine/g wet weight was found in a rapidly dividing population of nematodes.

Significance to Cancer Research: This research project has produced a number of new techniques (documented in this and previous reports) for study of gene expression in mammalian cells and thus has made an important contribution to basic cell biology. Ultimately, a more thorough understanding of gene expression in mammalian cells will provide guides to control of malignant growth and thus will provide new insight into practical cancer therapy and prevention.

Honors and Awards: None

Publications:

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5217

Title: Study of mechanisms of information transfer between cells of nervous system origin

Principal Investigator: Samuel H. Wilson

Section: Biosynthesis Section

Lab/Branch: Laboratory of Biochemistry

Goal: To develop techniques and procedures for investigation of communication between cells in the nervous system.

Approach: Uncloned neuroblastoma C-1300 cells were grown in surface culture, and tested for their content of the putative neurotransmitter, histamine. Amounts of histamine were determined using a new assay based on the principle of isotope dilution.

Progress: An assay for the histamine content of cultured cells was developed, and applied to determine that neuroblastoma cells contain a high level of histamine. The histamine content of the free-living nematode, C. elegans, was also recovered.

Significance for Cancer Research (NCP Objective 3 Approach 2)

This research project has produced several important techniques for the study of gene expression in mammalian cells. A more thorough understanding of gene expression in mammalian cells will provide guides to the control of malignant growth.

Level of Effort: Professional Man-Years: 1/8

Serial No. NCI-5210

1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

NIH-PHS
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Control of gene expression and growth in animal cells

Previous Serial No.: Same

Principal Investigator: E. Brad Thompson

Other Investigators: D. Aviv, L. Lyons, E. L. Kuff and S. H. Wilson

Cooperating Units: Drs. M. Lippman and S. Perry, Chemotherapy Branch, NCI; D. Nebert, W. Benedict and I. Owens, LBS, NICH; M. Feigelson, Dept. of Endocrinology, Columbia Univ. College of Physicians and Surgeons; S. S. Yang, LCB, NCI; E. Henderson, B. Levinthal and R. Halterman, Med. Branch, NCI; A. Hershko, Dept. of Biochemistry, Aba Khovsky School of Medicine, Haifa, Israel; H. Aviv, R. Callahan and P. Leder, LMB, NICH.

Man Years:

Total: 10

Professional: 7

Other: 3

Project Description:

Objectives: To continue our pursuit of the basic cellular controls of gene expression and growth in normal and tumor cells. To apply this information where possible to clinical situations.

Methods Employed: In addition to the methods cited in previous reports, we are developing the capacity to carry out chromosomal banding techniques. We have also added new methods of mRNA purification by affinity chromatography and improved competitive binding assays for steroid receptors.

Major Findings:

1) Growth Control Using Cell Hybrids. We have repeated our experiments fusing HTC cells and L cells and confirmed that such hybrids may display contact inhibition, whereas the parents do not. We are setting up studies of the

biology and biochemistry of this event.

Other hybrids, between 3T3 and HTC cells are being examined for control of growth and related macromolecular responses as well, in a pilot study with Dr. Hershko.

2) Control of Gene Expression Using Cell Hybrids. A) We are continuing our enzyme-control studies in hybrids between HTC cells and 3T3 cell variants in aryl hydrocarbon hydroxylase. B) Studies of steroid receptors are underway in hybrids between various cells inhibited or enhanced by steroids. These hybrids show considerable promise as clues to how steroids act on cells.

3) Cell-Free Protein Synthesis Studies continue studies completed with Drs. Wilson and Kuff in the inhibition of globin synthesis by polymers of deoxythymidylate showed the inhibition to occur at initiation of peptide chains. The inhibition could be partially reversed by crude cell supernatant or ribosomal washes but not by additional mRNA. In new studies, preliminary results suggest that the steroid-inducible enzyme, tyrosine aminotransferase may be identified as a translation product.

4) Spontaneous Cell Variants of the HTC Hepatoma Line showing high or low inducibility for tyrosine aminotransferase have been characterized more fully with regard to biochemical and physiological parameters. The transaminase of the non-inducible cells seems identical with that of the inducible lines. Both possess steroid receptors; however, in the non-inducible lines the steroid-receptor complex appears to fail to concentrate in the nucleus.

5) In Human Leukemias, Steroid Receptors have been studied. In acute lymphoblastic leukemia, the presence of receptors correlates with the clinical¹ and in vitro response to therapy which includes glucocorticoids. This correlation suggests that the response of a patient to glucocorticoid therapy may be predicted by a simple test on peripheral blood, and a decision made whether to treat with steroids. In addition, these are the first demonstration of a correlation between glucocorticoid receptors and biological response in a human tissue.

6) A Serum-Free Cell Line has been developed from the HTC cells, and its growth requirements are being determined.

7) Preliminary Results suggest that histidase is induced by steroids in certain hepatomas.

8) Pilot Studies are being done studying the effect of rifampicin derivatives on growth control in transformed cell lines.

Significance to Cancer Research: We are continuing our attempt to study gene expression utilizing both cell biology and biochemistry. Therefore, we work to develop useful cell variants and specific cell-free systems as well. We believe that combining these two approaches will be fruitful in understanding gene regulation, growth control and cancer.

We have been able to take advantage of some of our background knowledge in the studies on steroid receptors in leukemia. Therapeutic decisions based on these types of biochemical data may be possible, with improved survival times resulting.

Proposed Course of Research: We shall pursue the following directions:

- 1) Development of additional mutant cell lines.
- 2) Analysis of growth control and steroid responses in hybrid cells.
- 3) Studies on steroid receptors and biochemical responses to steroids.
- 4) Cell-free synthesis of proteins.
- 5) Prospective study of steroid receptors in leukemias.
- 6) Attempt to develop steroid-receptor studies in other human tumors if facilities permit.
- 7) Attempt to develop cell-free transcription system which is steroid-sensitive.

Honors and Awards: Invited lecturer: 2nd Annual Alza Research Conference, held in Yosemite, Calif. ; NIH lectures in Biochemistry; UCLA Radiation Therapy Labs, Cancer Research Institute, Columbia College of Physicians and Surgeons; Univ. of Florida Medical School; Univ. of Maryland Dental School, Dept. of Biochemistry; Advanced Topics in Endocrinology, NIH.
Organized Fogarty International Center Meeting on Control of Gene Expression in Tissue Culture Cells, held April 4-6, 1973, NIH.

Publications:

1. Miller, J. V. Jr., and Thompson, E. B.: Radioactive assay for tyrosine aminotransferase. Anal. Biochem. 47: 487-494, 1972

2. Miller, J. V. Jr., Cuatrecasas, P., and Thompson, E. B.: Purification of tyrosine aminotransferase by affinity chromatography. Biochim. Biophys. Acta 276: 407-415, 1972.
3. Miller, J. V. Jr., Thompson, E. B., Kuff, E. L., and Wilson, S. H.: Polydeoxythymidylate inhibition of rabbit reticulocyte RNA dependent protein synthesis in a Krebs II ascites cell system. Biochem. Biophys. Res. Commun. 48: 1280-1286, 1972.
4. Aviv, D., and Thompson, E. B.: Variation in tyrosine aminotransferase induction in HTC cell clones. Science 177: 1201-1203, 1972.
5. Miller, J. V. Jr., Wilson, S. H., Kuff, E. L., and Thompson, E. B.: Inhibition of cell-free globin synthesis by polydeoxythymidylate. Biochim. Biophys. Acta 294: 507-516, 1972.
6. Levisohn, S. R., and Thompson, E. B.: Gene expression and contact inhibition in HTC/L cell hybrid lines. J. Cell Physiol. In press.
7. Thompson, E. B.: Temporal aspects of macromolecular synthesis in eukaryotic cells. In Urquhart, J. (Ed.): Temporal Organization in Living Systems Monograph, Proc. of the 2nd Alza Research Conference, 1972. New York, New York, Pergamon Press, In press.
8. Thompson, E. B., and Miller, J. V. Jr.: Enrichment of polysomes synthesizing a specific protein by use of affinity chromatography. In Colowick, S. P. and Kaplan, N. L. (Eds.): Methods in Enzymology Hormones and Cyclic Nucleotides. New York, New York, Academic Press, In press.
9. Lippman, M., Thompson, E. B., Halterman, R., Leventhal, B., and Perry, S.: Glucocorticoid binding proteins in human leukemic lymphoblasts. Nature New Biology 242: 157-158, 1973.
10. Lippman, M., Halterman, R. H., Leventhal, B. G., Perry, S., and Thompson, E. B.: Glucocorticoid binding proteins in human acute lymphoblastic leukemic blast cells. J. Clin. Invest. In press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5210

Title: Control of gene expression and growth in animal cells

Principal Investigator: E. Brad Thompson

Section: Biosynthesis Section

Lab/Branch: Laboratory of Biochemistry

Goal: To combine cell biology and biochemistry to study gene and growth control.

Approach: Studies on the mechanism of action of steroid hormones in tissue culture are showing promise of revealing general facts about gene expression in animal cells. Biochemical systems are being developed to study in detail the steroid-controlled synthesis of specific cell products, for example, the enzyme tyrosine aminotransferase. Steroid receptor proteins are being studied for their role in controlling cell phenotype.

An application of this work has been the study of steroid receptors in human leukemia.

Progress: In human acute lymphoblastic leukemia, response to therapy including glucocorticoids has been found to correlate with the presence of steroid receptors in the leukemic blasts.

Cell hybrids between L cells and HTC cells have been found to display controlled growth in contradistinction to either parent.

Gene expression in cell hybrids and in spontaneous HTC cell variants has been studied.

A serum free HTC cell line has been developed.

Significance for Cancer Research (NCP Objective 3 Approach 1)
Treatment of leukemia may be altered in certain patients according to tests for their steroid receptors. Basic information on control of growth and gene expression is fundamental to cancer research.

Level of Effort: Professional Man-Years: 7

Serial No. NCI-379

1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Ultrastructural characterization of immunoglobulins, enzymes and other biomacromolecules

Previous Serial No.: Same

Principal Investigator: Emma Shelton

Other Investigators: T. Deuel, Dept. of Medicine, Univ. of Chicago
L. Steiner, Dept. of Biology, Mass. Inst. of Technology
T. Gessner, and S. R. Himmelhoch, Lab. of Biochemistry

Cooperating Units:

Man Years:

Total: 3

Professional: 2

Other: 1

Project Description:

Objectives: To relate the fine structure of macromolecules to their biochemical and physical properties, and to their function in biological systems.

Methods and Major Findings:

1) In collaboration with T. Gessner and R. Himmelhoch, it was possible to isolate in very high purity the granules from guinea pig eosinophil polymorpholeukocytes. Granules were released from the cells by lysis in heparinized sucrose and after removal of the released DNA on a glass bead column, the granules were sedimented at 39,000 g. The yellowish pellet was found by electron microscopy to be composed almost exclusively of eosinophil granules. It was found further by electron microscopy that the crystalloid characteristic for the eosinophil granule was a discrete entity capable of being separated from the granule matrix by treatment with Triton X-100. The proteins of the granules were completely extracted with cetyltrimethylammonium bromide and examined by polyacrylamide gel electrophoresis. Four major and several minor proteins, all of them basic, were resolved. One of the major bands was identified as eosinophil peroxidase.

2) In collaboration with T. Deuel and D. G. Roth, the purification and regulatory properties of the enzyme, phosphoribosylpyrophosphate synthetase, are being studied. The enzyme, when extensively purified from rat liver, appears to occur in large molecular weight complexes and upon electron microscopy, the individual molecules can be seen to stack in long, linear aggregates. The substructure of

the molecule and the effect of stacking upon enzyme activity is being studied.

3) In collaboration with L. Steiner, the structure of several amphibian macroglobulins has been studied. A new finding is the observation that the macroglobulin of the bullfrog, *R. catesbiana*, is a tetrameric molecule. We have

Significance to Cancer Research: A knowledge of the structure of macromolecules obtained by observations with the electron microscope provides in many cases an unequivocal basis for a knowledge of their function as well as an unequivocal confirmation of physical-chemical data. Thus, we have shown that the complement component, Clq, has a flexible structure that would permit conformational change upon interaction with antigen-antibody complexes.

Proposed Course of Research: To elucidate further the structure of enzymes and immunoglobulins and correlate their structure with their function.

Honors and Awards: None

Publications:

1. Desser, R.K., Himmelhoch, S.R., Evans, W.H., Januska, M., Mage, M. and Shelton, E., Guinea pig heterophil and eosinophil peroxidase. Arch.Bio-chem.Biophys. 148, 452-465, 1972.
2. Shelton, E., Yonemasu, K. and Stroud, R.M.; Ultrastructure of the human complement component, Clq. Proc.Nat.Acad.Sci. USA 69: 65-68, 1972.
3. Acton, R.T., Weinheimer, P.F., Shelton, E., Niedermeier, W. and Bennett, J.C.: Phylogeny of immunoglobulins: Purification and physico-chemical characterization of the immune macroglobulin from the turtle, Pseudemus, scripta. Immunochemistry 9: 421-433, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-379

Title: Ultrastructural characterization of immunoglobulins, enzymes and other biomacromolecules

Principal Investigator: Emma Shelton

Section: Biosynthesis Section

Lab/Branch: Laboratory of Biochemistry

Goal: To relate the fine structure of macromolecules to their biochemical and physical properties and to their function in biological systems.

Approach: Examination of negative-stained purified individual macromolecules and interacting macromolecules in the electron microscope and interpreting the resulting images.

Progress: 1) Monitoring the isolation of eosinophil granules with the electron microscope has permitted identification of granule proteins. 2) Certain structural characteristics of phosphoribosyl pyrophosphate synthetase have been studied. 3) The fine structure of amphibian immunoglobulins has been studied.

Significance for Cancer Research (NCP Objective 5 Approach 6)

A knowledge of the structure of macromolecules obtained by observations in the electron microscope provides in many cases an unequivocal basis for a knowledge of their function as well as unequivocal confirmation of physico-chemical data.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-5215
1. Laboratory of Biochemistry
2. Biosynthesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biological and ultrastructural studies on cell cooperation in the immune response

Previous Serial No.: Same

Principle Investigator: Emma Shelton

Other Investigators: W. I. Bennett and M. G. Mage

Cooperating Units: None

Man Years:

Total: 3

Professional: 2

Other: 1

Project Description:

Objectives: To examine the interaction of lymphocytes and macrophages cooperating in the immune response in order to answer questions relating to:

- A) the role of the macrophage in the immune response.
- B) localization of antigen and antibody in cooperating cells.
- C) the types of lymphocytes (thymus-dependent or bone marrow-dependent) responding to various antigens.

Methods and Major Findings:

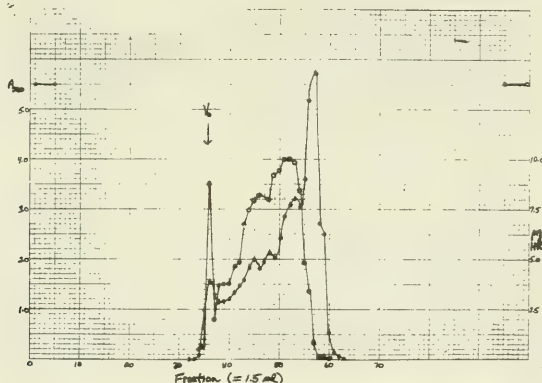
A) As part of the larger question concerning the presentation of antigen to lymphocytes by macrophages, there is conflicting evidence concerning the smaller question, "Can macrophages retain antigen on their exterior surface (plasma membrane) for long periods of time, i.e., days rather than minutes?"

We are preparing to explore the latter question by looking at antigen retained on cell surfaces with the electron microscope. To this end, we have prepared purified anti-horseradish peroxidase antibody and labeled it with ferritin-labeled antibody that is specific for that antigen. Rabbits were hyperimmunized against HRP and the gamma globulin fraction of rabbit whole serum was used as starting material for the preparation of specific antibody.

Cyanogen bromide-activated agarose beads (Sephacrose, 4B), allowed to react with HRP (Sigma, Type VI) overnight will bind better than 95% of the HRP protein. The anti-HRP antibody was purified by mixing whole rabbit gamma globulin with the beads, pouring a short column, washing it free of non-specific gamma globulin and eluting the antibody attached to the HRP bound to the beads.

Initial attempts to follow standard protocols for the above procedure resulted in total loss of specific antibody. It was, therefore, necessary to explore a variety of conditions in order to find a way of separating the antibody from the HRP that was bound to the column. It was found that successful purification of the antibody could be accomplished by eluting it from the column with 0.1 M Na_2PO_4 , pH 12. A drawback of the method was the disturbance of flow rate during the elution caused by slight denaturation of the agarose at this pH. Despite its drawbacks, the method has resulted in a 6 to 7-fold purification of antibody with a 50% yield of total antigen-binding capacity. The minimum estimate of the purity of such a preparation is 80%.

Extensively purified ferritin was coupled to the antibody by the toluene diisocyanate reagent of Singer, et al. and the resulting product was purified by agarose gel filtration. From antigen-binding estimates performed on the pooled fractions, it was estimated that each ferritin molecule was associated with one or two antigen-binding sites on the antibody.



A typical elution pattern of ferritin-labeled antibody from an agarose column is shown above. Protein concentration, measured by absorbancy at 280 μm (solid circles). Amount of horseradish peroxidase precipitated by 50% saturated $(\text{NH}_4)_2\text{SO}_4$ (open circles). Aggregated material appears in the void volume. The principle conjugate peak, fractions 49-54, precedes the main, unconjugated ferritin peak.

B) In the last annual report we outlined the rationale for the use of HRP as an antigen and the use of mouse peritoneal cells growing in diffusion chambers as a model system for observing the interaction of antibody-producing cells. During the past year, we have been able repeatedly to demonstrate the presence of anti-HRP antibody within plasma cells that have appeared in diffusion chamber cultures of immune mouse peritoneal cells exposed to HRP. It has not been possible to demonstrate any specific interaction between cells that could be related to the

appearance of antibody in the plasma cells. It is puzzling that while individual plasma cells are filled with antibody, there is very little evidence of the formation of a cluster or clone of antibody producing cells and antibody can seldom be detected in the chamber fluid or serum of the host animal. We postulate that this has something to do with the nature of HRP as an antigen of small molecular weight. We do not know yet whether macrophages are required for the formation of anti-HRP antibody by peritoneal cells.

Plating experiments. In order to look at the very early events during antigen processing, we have devised a technique termed "plating technique" whereby 2 or 3 microliter droplets containing $20-30 \times 10^5$ peritoneal cells are allowed to settle on thin millipore filters (0.45 μ m pore size, 25 μ m thick, 6 mm in diameter) supported on thick millipore filters (1.2 μ m pore size, 150 μ m thick, 47 mm diameter) which have been soaked in 0.4 ml of 20% mouse serum in phosphate buffered saline. Cells in such preparations can be maintained for 1 hour in a moist CO₂ 37°C incubator with no visible deterioration. Cells are mixed with HRP, incubated, fixed stain histochemically for HRP and with toluidine blue for cell morphology and mounted on slides. It is possible clearly to delineate which cells are "labeled" with HRP. In initial experiments, it was plain that HRP was rapidly taken up by immune macrophages (100% labeled at 15 min.) and large lymphocytes (80% labeled) while only 1-2% of lymphocytes were labeled. In contrast, only 25% of non-immune macrophages were labeled and no non-immune lymphocytes of either class was labeled. Subsequent experiments showed that these results were due entirely to the presence of antibody in the peritoneal fluid of the immune animals and when antibody even in low concentrations (4 μ g/ml) was added to non-immune cells, their labeling with HRP was identical to that of immune cells.

Since the phenomenon is dependent upon the presence of antibody, it is clear that we are looking not at uptake of HRP by these cells but at interaction with antibody that is labeled with HRP. Since it is known that the cells that carry antibody receptors are macrophages and "B" lymphocytes, we are able to identify the large lymphocytes in our preparations as "B" lymphocytes. We are preparing to exploit the technique to characterize the "B" lymphocytes with electron microscopy and to follow their interactions with other cells.

C) Preparation of anti-theta antiserum. One way to study the type of lymphocyte that is responding to various antigens is to eliminate it or label it with cytotoxic antibody. We have prepared antiserum against the theta antigen carried by the thymus-dependent or "T" lymphocyte by injecting thymus cells from AKR (cum) mice which bear the theta_{C3H} antigen into AKR (jax) mice which bear the theta_{AKR} antigen. Since these mice are otherwise isogenic, we have a pure, potent AKR reagent at our disposal.

Significance to Cancer Research: An examination at the ultrastructural level of cells interacting in the immune response should lead to an expansion of our knowledge of how such cooperation is effected. An understanding of such cooperation has ultimate usefulness in the treatment of cancer and other diseases. Objective 3, Approach 4 of the National Cancer Plan.

Proposed Course of Research: By using tracers visible at the ultrastructural

level, we hope to follow (1) the interaction of macrophages and lymphocytes during the production of antibody in the secondary response of these cells while growing in diffusion chambers, and (2) to trace the distribution of antigen on macrophages and lymphocyte plasma membranes.

Honors and Awards: Associate Editor, Cancer Research

Publications: Not yet

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5215

Title: Biological and ultrastructural studies on cell cooperation in the immune response

Principal Investigator: Emma Shelton

Section: Biosynthesis Section

Lab/Branch: Laboratory of Biochemistry

Goal: To examine (1) antigen receptors on cell surfaces and (2) follow plasma cell kinetics

Approach: 1) Freeze-dry shadow and replication techniques combined with electron microscopy to follow distribution and retention of antigen on cell surfaces. 2) To follow differentiation of mouse peritoneal cells into plasma cell after exposure to specific antigen in diffusion chambers.

Progress: 1) Ferritin labeled antibody specific for horseradish peroxidase (HRP) antigen has been prepared for use in locating antigen on cell surfaces. 2) Antibody receptors on macrophage and "B" lymphocytes surfaces can be followed with HRP-antibody complexes. 3) Mouse peritoneal cells in response to specific antigen will differentiate into plasma cells within 72 hours in diffusion chambers.

Significance for Cancer Research (NCP Objective 3 Approach 4)

An examination at the ultrastructural level of cells interacting in the immune response should lead to an expansion of our knowledge of how such cooperation is effected. An understanding of such cooperation has ultimate usefulness in the treatment of cancer.

Serial No. NCI-380
1. Laboratory of Biochemistry
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Deoxyribose containing compounds in normal, regenerating
and cancer tissues

Previous Serial No.: Same

Principal Investigator: Walter C. Schneider

Other Investigators: William Kidwell, Gilbert Smith

Cooperating Units: Laboratory of Biology

Man Years:

Total: 3

Professional: 1

Other: 2

Project Description:

Objectives: Since the replication of DNA is an obligatory prerequisite for cell division, the study of DNA synthesis and its control occupies a position of unique importance in cancer research because the malignant cancer cell displays continuous and uncontrolled division. The objectives of this project are to isolate and identify deoxyribose-containing compounds other than DNA from normal, precancerous, and tumor tissues, to determine the metabolic sequences in which these compounds are involved and whether these sequences are the same in normal precancerous and cancer tissues; to isolate DNA from subcellular structures other than nuclei, and to study the physical and biochemical properties of extranuclear DNA and to determine the relationship between nuclear and extranuclear DNA in carcinogenesis.

These studies are aimed at uncovering differences between normal and malignant cells that can be exploited to prevent the formation of cancer cells or to promote the destruction of cancer cells already generated. (These studies support most closely Objective 3: To prevent conversion of cells to those capable of forming cancer and Approach 1: Nature and modification of the precancerous state, of the National Cancer Plan).

Methods: Nuclei, mitochondria, zymogen granules, and microsomes were isolated from rat or mouse tissues by differential and/or density gradient centrifugation as well as by centrifugation in the zonal rotor. Nucleic acids were extracted from tissue fractions using sodium lauryl sulfate and phenol. DNA was purified from these extracts by treatment with ribonuclease and passage through Biogel P-60, by banding in CsCl, or by treatment with NaOH to remove RNA and small molecules.

DNA samples were examined by means of analytical CsCl density gradient centrifugation, melting behavior, and amount of labeled thymidine incorporated. DNA was measured by a modification of the diphenylamine reaction.

Enzyme markers were used for estimating purity of isolated subcellular fractions. Reduction of INT (iodonitrotetrazolium violet) in the presence of succinate or DPNH was used as a measure of mitochondria or microsomes, respectively. Amylase was used as a measure of zymogen granules in the pancreas.

Major Findings:

Zonal Centrifugation. Although it was anticipated that this method for isolating subcellular fractions would be widely used during the year, technical difficulties either with the rotor itself or the gradient forming machinery prevented this. However, it was possible to perform enough runs with the rotor to show that pancreatic homogenates could be fractionated successfully to yield a highly purified zymogen granule fraction. This fractionation was highly reproducible in the Spinco B-29 rotor. A number of runs were also performed with the Sorvall SZ-14 zonal rotor. This rotor is inherently simpler to use than the B-29 and gave fractionation similar to those obtained with the latter, although the separations did not appear to be as sharp as those obtained with the B-29.

Cytoplasmic DNA. One of the inherent problems in studying cytoplasmic DNA, besides the small quantities involved, is the separation of the two types of DNA: mitochondrial and microsomal. We previously observed that microsomal DNA was associated with membranes after treatment of microsomes with deoxycholate and could be removed by centrifugation. We have now found that if isolated mitochondria and microsomes are treated with a mixture of Triton X-100, deoxycholate and NaCl, the mitochondrial DNA was solubilized while the microsomal DNA could be spun down. This finding therefore appeared to provide a way for separating the two types of DNA without requiring the isolation of the two types of particles. It also permitted a closer examination of the question whether microsomal DNA had leaked from the nucleus, since tissues could now be disrupted in a medium which preserved only nuclei. Accordingly liver was homogenized directly in 0.25M sucrose containing 0.5% Triton X-100 and 2 mM Mg^{++} and the nuclei were spun down at low speed. The supernatant fluid was then supplemented with

EDTA equivalent to the added Mg^{++} and with deoxycholate and NaCl. When this mixture was spun at high speed two types of DNA were obtained: one, soluble, and with a specific activity similar to that of mitochondrial DNA, and the other particulate, and with a specific activity similar to that of microsomal DNA.

Superovulated Ovaries. This tissue was chosen for a study of microsomal DNA because of a report in the literature of a marked increase in smooth endoplasmic reticulum in this organ in pregnancy or following hormone treatment. Biochemical studies of whole ovaries following treatment with pregnant mare serum and prolactin showed that there was an increase of 35-40% in endoplasmic reticulum as judged by DPNH-INT reductase activity. There was a much smaller increase in the specific activity of microsomal DNA under these conditions. Autoradiographs provided more important information, however. They showed that thymidine incorporation had occurred not only over nuclei and mitochondria but also over other parts of the cytoplasm, some of which could be identified as endoplasmic reticulum. Grain counts on luteal cells showed that grains over cytoplasm outnumbered grains over nuclei and that an enormous increase in cytoplasmic grain count occurred in response to prolactin treatment.

Significance of Findings: The present findings provided further evidence for the view that microsomal DNA was not an artefact resulting from nuclear leakage. This was indicated first of all by the experiments in which liver cells were disrupted directly in Triton X-100 containing medium. The strongest evidence for this conclusion came from the autoradiographs of the luteal cells of the ovary which showed the presence of many cytoplasmic grains not associated with mitochondria and therefore provided strong evidence for an extra-mitochondrial cytoplasmic DNA. Unfortunately, biochemical studies of the ovary did not show the dramatic differences observed with the autoradiographic method because these studies had to include other ovarian cells as well as the luteal cells.

Proposed Course of Research: The experiments on direct separation of mitochondrial and microsomal DNA will be continued. The zonal rotor will continue to be used for isolating zymogen and other granules and for determining whether these granules contain DNA.

Experiments are also planned using a better system than the ovary. These studies would use the mammary epithelial cell system developed by Topper and his associates. These cells are reported to have a several fold increase in endoplasmic reticulum in culture under suitable conditions of hormone supplementation and should provide a better tool for studying microsomal DNA.

Honors and Awards: None

Publications:

1. Schneider, W. C.: Methods for the isolation of particulate components of the cell. In Umbreit, W. W., Burris, R. H., and Stauffer, J. F. (eds.): Manometric and Biochemical Techniques, ed. 5. Minneapolis, Minn., Burgess Publishing Co., 1972, pp. 196-212.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-380

Title: Deoxyribose containing compounds in normal, regenerating and cancer tissues

Principal Investigator: Walter C. Schneider

Section: Nucleic Acids Section

Lab/Branch: Laboratory of Biochemistry

Goal: To isolate and identify cytoplasmic DNA and precursors of DNA from normal, precancerous and tumor tissues and to determine their role in the carcinogenic process.

Approach: Nuclei, mitochondria, zymogen granules and microsomes are isolated by differential or zonal centrifugation. DNA is extracted from isolated fractions by a phenol procedure and is purified by banding in CsCl, passage through Biogel P-60, or hydrolysis with NaOH to remove RNA and small molecules. DNA samples are characterized by the amount of ^3H -thymidine incorporated, by their base composition and by their reaction with diphenylamine. Isolated subcellular fractions are tested for purity by chemical composition and enzyme markers.

Progress: The isolation of zymogen granules in the zonal rotor has been tested further and found to be highly reproducible and to yield highly purified preparations in a single step. A method has been devised for the separation of mitochondrial and microsomal DNA from tissue homogenates without the isolation of the mitochondria and microsomes. It is based upon differential solubility in detergent solutions. Evidence for the existence of a microsomal associated DNA was obtained in luteal cells of the ovary using autoradiography after thymidine- ^3H injection. The amount of incorporation was stimulated by prolactin.

Significance for Cancer Research (NCP Objective 3 Approach 1)
Since the functions of mitochondrial and microsomal DNA's have not been established and the studies so far are limited mainly to normal cells, it cannot be decided whether these DNA's are involved in the carcinogenic process. Experiments to study these questions will be difficult because of the small amount of DNA involved.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-5205
1. Laboratory of Biochemistry
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on biochemical mechanisms of sensitivity and resistance to antimetabolites and mechanisms for control of nucleic acid synthesis

Previous Serial No.: Same

Principal Investigator: Elizabeth P. Anderson

Other Investigators: Alec S. Liacouras

Cooperating Units: F. K. Millar, Laboratory of Physiology, NCI

Man Years:

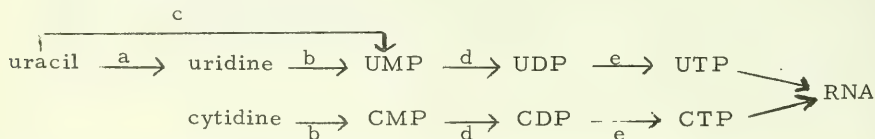
Total: 2 + 1-3/4 (not payroll) 3-3/4

Professional: 2 + 1-1/4 (not payroll) 3-1/4

Other: 1/2 (not payroll)

Project Description:

Objectives: The objective of this project is to investigate nucleotide metabolism in normal and cancer cells. The aim is to elucidate pathways of biosynthesis, mechanisms of control over these pathways, and biochemical mechanisms of sensitivity and resistance to purine and pyrimidine analogs. The end-products of nucleotide biosynthesis are the triphosphate precursors of nucleic acids, and regulation of the enzymes involved in these biosynthetic steps is probably a major factor in determining the rate of cellular nucleic acid synthesis. In view of the central role of UMP in pyrimidine metabolism, work has been focussed on reactions b and d in the following pathway of pyrimidine nucleotide biosynthesis:



Methods Employed and Major Findings:

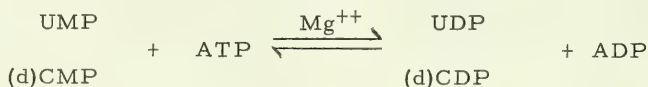
1) Studies on uridine-cytidine kinase. In mammalian tissues uridine kinase

(reaction b above) is the initial step in pyrimidine nucleotide biosynthesis from the preformed ring. This reaction has been shown to be rate-limiting in the biosynthetic pathway and susceptible to feedback inhibition by nucleoside triphosphate end-products. This enzyme has now been purified approximately 150-fold from acetone powder preparations of the mouse ascites mast cell neoplasm P815/S; this has been achieved using fractionation on DEAE-cellulose, ammonium sulfate fractionation, and separation on hydroxyapatite. The ratio of uridine to cytidine kinase activity remained constant through these purification steps, and the sensitivity to feedback inhibition by CTP and UTP was retained. Two assay methods are presently being used for this enzyme reaction; one measures the conversion of C¹⁴-labeled uridine to nucleotide product, while the second, which can only be used on more purified fractions, assays the production of ADP by coupling this to the pyruvate kinase - lactate dehydrogenase system and spectrophotometrically determining the conversion of NADH to NAD⁺. The purified enzyme has now been characterized in several respects. It exhibited a broad pH optimum around 6.5 and a divalent cation requirement which could be met most effectively by Mg⁺⁺ and to a smaller extent by Mn⁺⁺, Co⁺⁺, Fe⁺⁺, and Ni⁺⁺, in that order. Beside the substrates uridine and cytidine, only 5-fluorouridine and the 5-aza- and 6-aza-analogs of uridine and cytidine were phosphorylated by the enzyme; deoxyribonucleosides were not phosphorylated, nor was cytosine arabinoside. The enzyme has a somewhat broader specificity with regard to the phosphate donor; of the nucleoside triphosphates tested, ATP was the most active, with dATP nearly as active, and dUTP, dCTP, GTP, and dGTP showing decreasing activity. With either uridine or cytidine as phosphate acceptor, and ATP as donor, initial velocity studies using the continuous assay gave a pattern of intersecting lines, indicating that the mechanism of the reaction was one of sequential addition of both substrates to the enzyme. Analysis of the data by Cleland's "SEQUEN" computer program indicated a K_m for uridine = 0.15 mM, K_m for cytidine = 0.045 mM, and K_m's for ATP 3.5 and 2.1 mM with uridine and cytidine, respectively. These studies have been carried out by Mr. Alec Liacouras; two manuscripts are in preparation reporting these results, one on the purification and characterization of the enzyme, and a second on the initial velocity studies and the mechanism of the reaction.

In P815/FUR, a 5-fluorouridine-resistant subline of P815/S, resistance to the analog is associated with a decreased level of uridine kinase (activity in crude extracts equal to 10-20% of that found in extracts from the parent sensitive line). Studies are under way to explore possible differences between the uridine kinases from the two lines. Toward this end, uridine kinase has now been purified 20-30 fold from the resistant subline, using similar methods of DEAE-cellulose chromatography and fractionation with ammonium sulfate. The low level of kinase activity in the resistant subline required a modification of the radioactivity assay procedure to afford accurate detection of the activity.

Characterization studies are under way on the resistant-line enzyme. A prominent difference between the two enzymes is the lability of that from the resistant line to low ionic strength; this property precludes further purification of this enzyme on hydroxyapatite, since low ionic strength buffers are required. Further purification on Sephadex G-150 is now being attempted; in preliminary experiments several uridine kinase activities were separated, all very labile, so heterogeneity of the enzyme is a real possibility. These studies have been carried out by Dr. Jane M. Glick, currently a postdoctoral fellow in this laboratory.

2) Studies on UMP-CMP kinase. In earlier work in this laboratory (Ruffner and Anderson, J. Biol. Chem. 244: 5994, 1969) the enzyme that catalyzes the two reactions labeled d above was purified 300-fold from Tetrahymena pyriformis; this is a rapidly growing protozoan unable to synthesize the pyrimidine ring de novo and hence dependent on pyrimidine anabolism from the preformed ring. The purified Tetrahymena enzyme catalyzes the phosphorylation of UMP and CMP and, to a lesser extent, of dCMP, and it has recently been suggested (Anderson, The Enzymes Vol. IX, 1973, in press) that this enzyme may be the same as, or similar to, the dCMP kinase purified from calf thymus by Sugino et al. (J. Biol. Chem. 241: 961, 1966). This is apparently the first extensive purification for CMP-UMP kinase activity, and based on our results on the substrate specificity of the purified preparation, this enzyme has been renumbered, in the new 1972 Recommendations on Enzyme Nomenclature of the Commission on Biochemical Nomenclature, as 2.7.4.14, ATP:(d)CMP phosphotransferase. The reaction catalyzed is:



Using a refined radioactivity assay to measure the phosphorylation of C^{14} -UMP, this enzyme has been investigated with regard to reaction mechanism. From data on isotope exchange in the partial reactions, it was concluded that this reaction also proceeds by a sequential mechanism and that a ternary complex of the enzyme with both substrates is formed as the central intermediate in the reaction. Initial velocity studies, varying both UMP and ATP concentrations, also supported this reaction mechanism; the pattern also indicated that the reaction is subject to competitive bisubstrate inhibition, suggesting similarity of the binding sites for these two substrates. These results have just been published in *Biochimica et Biophysica Acta*.

Investigations of the kinase reaction were undertaken with CMP as the phosphate acceptor, using the continuous spectrophotometric assay that

couples the production of ADP to the pyruvate kinase - lactate dehydrogenase system. With this substrate a less complicated initial velocity pattern was observed, and the evidence for a sequential reaction mechanism was even more clear-cut. When analyzed by Cleland's "SEQUEN" program, the data indicated K_m for CMP = 0.81 mM and K_m for ATP = 0.23 mM. The mechanism of the reaction has now also been investigated in the reverse direction, starting with the nucleoside diphosphates. The reaction is freely reversible, but the rates suggest that, physiologically, the enzyme is intended to function primarily in the forward or anabolic direction. The initial velocity pattern for the reverse reaction appears to be, again, one of intersecting lines, indicating that the reaction in both directions is indeed one of sequential addition of the two substrates to the enzyme. All evidence so far points to this addition as occurring in a random order, and the rate-limiting step in the reaction mechanism is very likely the interconversion of the enzyme-bisubstrate ternary complex. These results with CMP are now being prepared for publication.

The studies on this enzyme have been carried out by Dr. Thomas Garvey, who has continued with us this year as a ^{part-time} guest worker. In addition to the biochemical studies, he has made use of the IBM 1620, the IBM 360, and the PDP-10 computers for routine calculations and for the more complicated problems of curve fitting, statistical evaluation of the data, and calculation of the K_m 's and rate constants. In these computer operations, he has collaborated with Dr. F. K. Millar, Laboratory of Physiology, NCI. Dr. Garvey and Dr. Millar have also derived a mathematical expression which describes, at different pH's, the partitioning of Mg^{++} ion among various species in solution, such as ATP and phosphate, that have an affinity for the ion. This expression has been derived from the dissociation constants of the various Mg-complexes. They have programmed this for computer use; this method will be published and should enable an investigator to make an accurate calculation of the concentration of any particular Mg-complex in a mixture of species in solution.

3) Related activities. Work has been completed on a major review of nucleoside and nucleotide kinases for the new edition of The Enzymes. This covers both of these enzymes on which research is currently in progress as well as fourteen other related nucleoside kinases and nucleoside monophosphate kinases. This is now in press and will be published shortly.

The laboratory now has one STRIDE trainee, Mr. Harry Free. In addition to his course work at American University, Mr. Free has helped out very much in various aspects of our laboratory research. He has also begun a small research project of his own to follow the levels of UMP kinase activity at different stages of cell growth.

Significance to Cancer Research: UMP is the central compound in pyrimidine nucleotide biosynthesis in that it is the precursor for synthesis of other pyrimidine nucleotides of both the ribo- and deoxyribo- series. UMP synthesis by uridine kinase is called into play in rapidly growing tissues such as tumor tissues, and this constitutes a difference between normal and cancer cells. It is important to discover how the various enzymes for UMP synthesis and metabolism are regulated in both types of cells, since such regulation undoubtedly helps to maintain a balance among the levels of the various nucleotide precursors of nucleic acids and functions in regulating the rate of cellular nucleic acid synthesis.

Proposed Course of Research: The purification of uridine-cytidine kinase from the fluorouridine-resistant subline of the P815 mast cell tumor will be continued, and this enzyme will be compared to that from the sensitive line with respect to reaction mechanism and the various parameters of characterization, especially quantitative capacity to phosphorylate the natural nucleosides and fluorouridine. The two purified enzymes will also be compared with regard to molecular weight and possible subunit structure, and the intriguing possibility of different molecular species of the kinase will be explored. The mechanisms of feedback inhibition of this enzyme will also be investigated in terms of the nature of the enzyme molecule and its catalytic and regulatory sites.

The UMP-CMP kinase reaction will be explored further in the reverse direction, starting with the nucleoside diphosphates, and attempts will be made to establish definitively whether the addition of substrates to the enzyme is random sequential or ordered. At the completion of Dr. Garvey's studies with this enzyme in the near future, no further investigation of this enzyme is now planned, but the problem is open to subsequent exploration of the promising lead on the effect of thiol in allowing this enzyme to phosphorylate dCMP as well as CMP and UMP. This could be a mechanism by which the cell determines whether the ribonucleotide or deoxyribonucleotide is phosphorylated and built into nucleic acids.

Honors and Awards: Invitation to contribute chapter to The Enzymes

Publications:

1. Garvey, T. Q. III, Millar, F. K., and Anderson, E. P.: Adenosine triphosphate: uridine monophosphate - cytidine monophosphate phosphotransferase from Tetrahymena pyriformis. II. Kinetic studies and reaction mechanism with UMP. Biochim. Biophys. Acta 302: 38-49, 1973.

2. Anderson, E. P.: Nucleoside and nucleotide kinases. In Boyer, P. D. (Ed.): The Enzymes. New York, New York, Academic Press, 1973, Vol. IX, in press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5205

Title: Studies on biochemical mechanisms of sensitivity and resistance to antimetabolites and mechanisms for control of nucleic acid synthesis

Principal Investigator: Elizabeth P. Anderson

Section: Nucleic Acids Section

Lab/Branch: Laboratory of Biochemistry

Goal: To investigate nucleotide metabolism in normal and cancer cells in terms of enzymatic pathways, mechanisms of control over these, and biochemical mechanisms of sensitivity and resistance to purine and pyrimidine analogs.

Approach: UMP synthesis by uridine kinase is called into play in rapidly growing tissues. In view of the central role of UMP in pyrimidine metabolism, current work is focussed on UMP synthesis by this kinase and its phosphorylation by UMP kinase. Classical methods of enzymology are being used to purify and characterize both of these enzymes, to elucidate the mechanism of the reaction catalyzed in each case, and to explore the physiological role of these enzymatic reactions in the growing cell. Uridine kinase is the rate-limiting step in the anabolism of preformed pyrimidines and is susceptible to feedback inhibition by nucleoside triphosphate end-products. This enzyme is being examined in a mouse mast cell tumor and in a fluorouridine-resistant subline of this tumor, in which resistance to the analog is associated with markedly decreased levels of the enzyme. Differences between the two enzymes at the molecular and functional level are being sought, and the mechanism of the feedback regulation is being investigated. UMP kinase is being studied in another rapidly growing cell system, and the factors that determine the anabolic and catabolic capacity of this enzyme are being explored.

Progress: Uridine-cytidine kinase has been purified 150-fold from the mouse mast cell tumor and characterized as to pH optimum, divalent cation requirement, reaction mechanism, and specificity for both phosphate donor and acceptor. The enzyme phosphorylates various pyrimidine nucleoside analogs with anticancer activity. The reaction mechanism is apparently one of sequential addition of both substrates to the enzyme to form a ternary complex. This enzyme has been purified about 30-fold from the FUR-resistant subline; the preparation is very labile, and the possibility of different molecular species is being explored. UMP-CMP kinase has been purified 300-fold from growing Tetrahymena. Initial velocity patterns and isotope exchange in the partial reactions indicate that the mechanism of this reaction is likewise sequential.

INTRAMURAL RESEARCH SUMMARY CONTINUED

The rates suggest that, physiologically, the enzyme is intended to function primarily in the forward or anabolic direction.

Significance for Cancer Research (NCP Objective 3 Approach 2)

UMP is the central compound in pyrimidine nucleotide biosynthesis, the precursor of other pyrimidine nucleotides of both the ribo- and deoxyribo-series. Extensive UMP synthesis by uridine kinase is characteristic of cancer cells. It is important to discover how the enzymes for UMP synthesis and metabolism are regulated in normal and cancer cells, since such regulation undoubtedly helps to control the rate of cellular nucleic acid synthesis.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-5211
1. Laboratory of Biochemistry
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Initiation and maturation phenomena in the replication of mammalian DNA

Previous Serial No.: Same

Principal Investigator: William R. Kidwell

Other Investigators: R. E. Wittes and R. A. Colyer

Cooperating Units: None

Man Years:

Total: 3-1/2

Professional: 2

Other: 1-1/2

Project Description:

Objectives: The cells in most tissues and organs of higher species accumulate in a non-DNA replicative stage of the cell cycle denoted as G_0 or extended G_1 . In malignancy there is characteristically an increase in the rate of cell proliferation and the normal regulatory mechanism leading to an accumulation of cells in G_0 is lost. This investigation places emphasis on elucidating the mechanism of initiation of DNA synthesis, a rate limiting process in normal cells which occurs with accelerated frequency in malignancy.

One approach utilized is to characterize the initiation and replication of a special class of DNA in the nucleus of L-cells, satellite DNA, which by virtue of its reassociation properties and buoyant density can be separated from the bulk DNA of the nucleus. A second approach is to delineate cell cycle changes in the synthesis of a novel chromatin associated polymer, Poly Adenosine Diphosphate-Ribose, whose synthesis is reported to depress DNA replication in isolated nuclei, thus acting as an anti-initiator.

Methods Employed: To study the initiation phenomenon, an in vitro DNA synthesizing system utilizing mouse L-cells nuclei was developed. The

The differential affect of cytoplasmic extracts of cells at various stages of S on satellite and bulk DNA synthesis was assessed by fractionation of the two DNA species on hydroxyapatite following denaturation and reassociation.

Standard methods for cell synchrony included accumulation of cells in G₁ by growth to confluency followed by subculturing into shake culture in the presence of hydroxyurea to block cells at the G₁/S boundary. S-phase was initiated by the replacement of the blocking medium with fresh, hydroxyurea free medium after a suitable interval.

Results: Satellite DNA Synthesis: Earlier work on the synthesis of this DNA species in whole cells suggested that satellite DNA was replicated during the first two hours of S with a second period of synthesis in late S. This interpretation was based on the assumption that both satellite and bulk nuclear DNA is synthesized from the same TTP precursor pool. This assumption has been supported by the results of experiments conducted with R. E. Wittes. Randomly growing cells were pulse-labelled with ³H-thymidine for various times (the shortest time being less than the time required for equilibration of the TTP pools with ³H-thymidine from the medium) and nuclear DNA was isolated and separated into satellite and bulk DNA fractions. The relative incorporation of counts into satellite DNA versus bulk DNA was constant regardless of the length of the pulse. Since 10 percent of the total radioactivity incorporated is in satellite DNA and since satellite DNA constitutes 10 percent of the total nuclear DNA, it is unlikely that separate TTP pools exist for the synthesis of the two types of DNA.

Further characterization of the TTP pool size by means of kinetic analysis of ³H-thymidine accumulation in DNA revealed that the size of the TTP pool of cells utilized for DNA replication is of the same order of magnitude as the total TTP pool size in the cell. Therefore, the existence of a small, separate TTP pool for the replication of DNA as suggested by Friedman is untenable and in all probability, the total TTP pool of S-phase cells is utilized for DNA replication.

Poly Adenosine Diphosphate-Ribose: A postulated anti-initiation factor is a novel chromatin bound polymer, Poly ADP-ribose. Nuclei pre-incubated with NAD are rendered incompetent for TTP incorporation into DNA, presumably as a result of the synthesis of poly ADP-ribose from NAD (Biochem. Biophys. Res. Comm. 40: 1013, 1971). R. A. Colyer and I have investigated the synthesis of poly-ADP-ribose in intact L-cells utilizing ³H-adenosine. The in vivo synthesized polymer is localized almost exclusively in the nucleus, and is not labelled when cells are incubated with ¹⁴C-thymidine or ¹⁴C-uridine. It resists digestion with 0.3 M NaOH for 1 hour at 37° C. Degradation of the polymer with venom phosphodiesterase produces fragments which migrate

chromatographically as do the degradation products of authentic poly ADP-ribose. Pulse labelling experiments reveal two bursts of polymer synthesis at 1-2 and 3-4 hours into the S-phase of synchronized cells. Nicotinamide and thymine, which block poly ADP-ribose synthesis in vitro, also inhibit the ^3H -adenosine incorporation seen at 1-2 and 3-4 hours of S. These inhibitors of poly ADP-ribose polymerase have no effect on ^{14}C -thymidine incorporation during the first 2 hours of S but produce a 50 percent inhibition of DNA synthesis subsequent to the time of normal poly ADP-ribose synthesis. The results suggest that the synthesis of poly ADP-ribose, rather than its degradation is required for DNA synthesis in intact cells.

Significance to Cancer Research: Central to the question of increased cell proliferation in the malignant state is an understanding of the factors governing whether a cell will cease its progression through a subsequent cycle or whether it will traverse again through the G_1/S stage. That is, what governs primary initiation of replication?

The in vitro DNA synthesizing system utilized for studying initiation of satellite DNA suggests the following about initiation per se: (1) Initiation of replication is manifest by controls acting via the cytoplasm; (2) Signals for initiation appear in the cytoplasm just before or during the time of replication of a particular nuclear DNA species; (3) The appearance of these signals in the cytoplasm requires both protein and RNA synthesis; (4) Following the initiation event, there is a decay of the signal for initiation; or (5) Reinitiation is blocked by the interplay of negative control mechanisms (anti-initiation factors).

We have examined the synthesis during the cell cycle of one proposed candidate for the anti-initiation function, poly ADP-ribose. Although our examinations are still incomplete, they suggest that the synthesis of this polymer may actually promote rather than block DNA replication.

Proposed Course of Research: Continued effort will be directed toward elucidation of the phenomenon of satellite DNA initiation. In addition, emphasis will be placed on delineating the role of poly ADP-ribose and the relationship of its synthesis and degradation to DNA replication. Specifically, quantitation of the mass of the polymer, rather than the uptake of labelled precursor must be accomplished. In addition, the amount of poly ADP-ribose polymerase and the nuclear enzymes which degrade the polymer must also be quantitated.

Honors and Awards: Invitation to participate in Fogarty International Center Symposium on Poly-ADP Ribose, June, 1973.

Publications:

1. Kidwell, W. R., Saral, R., Martin, R. G., and Ozer, H. L.: Characterization of an endonuclease associated with purified SV-40 virions. J. Virol. 10 410-416, 1972.
2. Wittes, R. E., and Kidwell, W. R.: A kinetic approach to the determination of S-phase pool size of thymidine triphosphate in exponentially growing L-cells. J. Mol. Biol. In press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5211

Title: Initiation and maturation phenomena in the replication of mammalian DNA

Principal Investigator: William R. Kidwell

Section: Nucleic Acids Section

Lab/Branch: Laboratory of Biochemistry

Goal: This investigation places emphasis on elucidating the mechanism of initiation of DNA replication, a rate limiting process in normal cells which is accelerated in malignancy.

Approach: The approach utilized is to characterize the initiation and replication of a special class of DNA in L-cell nuclei, satellite DNA. Additionally, we are delineating the synthesis and turnover during the cell cycle of a novel, chromatin bound polymer, poly(ADP-ribose), whose synthesis is reported to depress DNA synthesis in isolated nuclei and thus may serve an anti-initiation function.

Progress: Experiments to date suggest that initiation per se is characterized as follows. Initiation is manifest by signals acting via the cytoplasm. These signals appear in the cytoplasm just before or during the time of replication of a particular nuclear DNA species, then the signals decay or anti-initiation factors develop. Accumulation of initiator signals require active protein and RNA synthesis. One postulated anti-initiator, poly ADP-ribose, has been studied throughout the cell cycle. It is synthesized predominantly during the S-phase and degraded as the cell progresses from G₂/M into G₁.

Significance for Cancer Research (NCP Objective 3 Approach 2)
Contral to the question of malignancy is an understanding of how cell proliferation is controlled. Elucidation of the factors involved in initiation of DNA synthesis which we are attempting may suggest new targets for a chemotherapeutic approach to the cancer problem.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-306

1. Laboratory of Biochemistry
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Studies of β -hydroxy- α -amino acids

Previous Serial No.: Same

Principal Investigator: Theodore T. Otani

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 2

Professional: 1

Other: 1

Project Description:

Objectives: To prepare chemical compounds which preferentially destroy cancer cells. The preparation of β -hydroxy- α -amino acids and their N-acylated derivatives is being attempted, since earlier experiments done in this laboratory indicated that some compounds of this group are capable of inhibiting bacterial as well as tumor growth.

More specifically, the immediate objectives of this portion of the project are: (a) To isolate the four isomers of β -hydroxynorleucine prepared in this laboratory, and also to prepare their respective N-acyl derivatives in sufficient quantities for study of their possible tumor inhibitory action in mammalian systems.

(b) To prepare and separate the isomers of the analogs of methionine, i.e., β -hydroxyhomomethionine, β -hydroxymethionine, and β -hydroxymethoxinine and their corresponding N-chloroacetyl derivatives.

(c) To prepare various halo-substituted aliphatic β -hydroxy- α -amino acids from halo-alkyl aldehydes and glycine copper complex.

(d) To prepare β -hydroxy- α -asparagine, and β -hydroxy- β -asparagine

from glycine amide and glyoxylic acid, and from glyoxylic acid amide and glycine respectively.

Methods Employed: The preparation of these compounds is carried out in alkaline medium with the appropriate aldehydes and glycine copper complex (or glycine and cupric ions), under conditions which are empirically determined.

Major Findings:

- 1) The purity of 3 of the 4 isomers of β -hydroxynorleucine, and of all 4 isomers of the N-chloroacetyl derivatives has been confirmed by: (a) paper chromatography in 5 solvent systems; (b) Van Slyke nitrous acid determinations of primary amino nitrogen; (c) elemental analysis; and (d) optical rotation determination.
- 2) The comparative rates of hydrolysis of the N-chloroacetyl derivatives of diastereomer A and diastereomer B of β -hydroxynorleucine have been determined. The rate of the latter is approximately twice that of the former.
- 3) Chloroacetyl derivatives of racemic β -hydroxyhomomethionine and of β -hydroxymethoxinine have been prepared. The isomers of the N-chloroacetylated β -hydroxyhomomethionine appear to be considerably less susceptible to the action of Acylase I than those of β -hydroxynorleucine. Of the 2 diastereomers of β -hydroxyhomomethionine, the N-chloroacetyl derivative of diastereomer A was found to be practically inert to the action of Acylase I (up to an enzyme concentration of 10 mg/ml), while the chloroacetyl derivative of diastereomer B showed some susceptibility to this enzyme.
- 4) Gram quantities of β -hydroxyhomomethionine and β -hydroxymethoxinine have been isolated in preparation for the separation of the 4 isomers of each compound. Also, the stockpile of the optically pure diastereomers of β -hydroxynorleucine and of their respective N-chloroacetyl derivatives has been increased.
- 5) By-products of β -hydroxyhomomethionine preparation have been identified tentatively as the corresponding sulfones of each diastereomer. This was accomplished by oxidation of the mother compound with H_2O_2 to yield the sulfone, which was then identified chromatographically by comparison with the compound appearing in the reaction mixture.
- 6) Trifluoroacetyl-DL-threonine has been prepared as a control compound for the synthesis of the trifluoroacetyl derivatives of other β -hydroxy- α -amino acids. the identification of its structure is now being determined.

Significance to Cancer Research: That amino acid metabolism may be a susceptible area of action in cancer therapy was demonstrated by the efficacy of asparaginase in certain tumors which required asparagine. Amino acid deprivation, either by the removal of the amino acid or by the substitution with an analog may be effective in inhibiting tumor growth.

The unnatural amino acids synthesized under this program are close structural analogs of essential natural amino acids, differing in some cases only in the presence of a single hydroxyl group adjacent to the α -amino group. Hence, β -hydroxynorleucine differs from norleucine only in the presence of a hydroxyl group in the β -position of carbon chain of the amino acid. β -Hydroxynorleucine is a structural analog of methionine, differing from the latter in having a methylene group in place of the sulfur atom in the γ -position of the chain. Indeed, β -hydroxynorleucine, even as a mixture of four isomers, not only exhibited inhibitory action in two microbial systems, but also showed some anti-tumor effect against Carcinoma 755 as measured by the decrease of tumor size and increase in survival time. β -Hydroxymethoxinine and β -hydroxymethionine are even closer analogs of methionine than is β -hydroxyhomomethionine, the former differing from methionine in possessing an oxygen atom in place of the S atom and in possessing a β -hydroxyl group, and the latter only in possessing a β -hydroxyl group.

The chain-halogenated amino acids, similarly, are close structural analogs of essential amino acids. For example, γ -dichlorothreonine would be an analog of leucine, since the size of the chlorine atom is approximately that of the methyl group; also γ -trifluorothreonine would be a close analog of threonine since the atomic radius of the fluorine atom is essentially that of hydrogen atoms.

Proposed Course of Research:

- 1) The isomers of both the free acids and of N-chloroacetyl derivatives of β -hydroxynorleucine, now confirmed to be optically pure will be tested initially on microbial anti-tumor screening system.
- 2) When sufficient quantities of these optically pure compounds are isolated they will be tested for anti-tumor activity in selected mammalian tumor screens.
- 3) Further chemical identification will be made and proof of structure will be sought for β -hydroxyhomomethionine and β -hydroxymethoxinine.
- 4) Attempts will be made to resolve the optical isomers of each diastereomer of β -hydroxyhomomethionine and of β -hydroxymethoxinine.

- 5) The corresponding N-acyl derivatives of each of the four optically pure isomers of each of these compounds will be prepared.
- 6) The optically pure diastereomers of the free acids as well as of the N-acyl derivatives will be tested for anti-tumor action in mammalian anti-tumor screens, and when sufficient quantities of each compound are made, they will be tested in mammalian tumor systems.
- 7) Preparation of N-trifluoroacetyl derivatives of these unnatural β -hydroxy amino acids will be attempted.
- 8) Attempts to prepare halo-substituted β -hydroxy amino acids, the β -hydroxy-asparagines (α - and β -), and N-acylated derivatives of other amino acid analogs will be made and these will be tested for anti-tumor activity.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-306

Title: Studies of β -hydroxy- α -amino acids

Principal Investigator: Theodore T. Otani

Section: Nucleic Acids Section

Lab/Branch: Laboratory of Biochemistry

Goal: To develop compounds that preferentially destroy cancer cells.

Approach: To design chemical compounds, which, when degraded by the metabolic action of the cancer cell (or by cells in close proximity of the cancer), release cytotoxic moieties which destroy the cancer cell.

Progress: Compounds which thus far appear to show moderate anti-tumor activity as measured by tumor growth inhibition and extension of survival time of host animals have been developed.

Significance for Cancer Research (NCP Objective 6 Approach 2)
To find new cancer chemotherapeutic agents with mode of action different from those presently being used.

Level of Effort: Professional Man-Years: 1

Serial No. NCI- 5221

1. Laboratory of Biochemistry
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Studies comparing the structure, properties and metabolism of the nucleic acids and nucleoproteins of normal rat and/or mouse tissues with comparable tissues that become neoplastic

Previous Serial No.: None

Principal Investigator: Jean Rotherham

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1 month

Professional: 1 month

Other: 0

Project Description:

Objectives: To discover if there are differences in the structure, properties and metabolism of the nucleic acids and nucleoproteins of normal rat and/or mouse tissues with respect to comparable tissues that are becoming neoplastic.

Methods Employed: Suitable chemical agents will be used to induce neoplastic changes in mouse and/or rat tissues. Nuclear fractions will be isolated and examined for possible differences in metabolic modifications of the nucleoproteins of tissues of treated and control animals.

Major Findings: This study is being initiated at this present time. Of two projects that were previously proposed (4/13/72, NCI-397 and NCI-5220), one, proposed for reactivation, was terminated because the biological material did not become available. After two months of experimental work to isolate chromatin from rat mammary tumors, the second project was terminated when it became apparent that supporting personnel required for the project would not be available.

Serial No. NCI-5221

Significance to Cancer Research: (See objectives). National Cancer Program
Plan Objective and Approach: Cause and prevention; nature and rate of
oncogenic cell transformations in carcinogenesis (3, 2).

Proposed Course of Work: This project is being initiated at this time.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5221

Title: Studies comparing the structure, properties and metabolism of the nucleic acids and nucleoproteins of normal rat and/or mouse tissues with comparable tissues that become neoplastic

Principle Investigator: Jean Rotherham

Section: Nucleic Acids Section

Lab/Branch: Laboratory of Biochemistry

Goal: To discover if there are changes in structure, properties or metabolism of the nucleic acids or nucleoproteins of rat and/or mouse tissues which are characteristic of and associated with neoplastic change.

Approach: Suitable agents will be used to induce neoplastic change in mouse and/or rat tissues. Nuclear fractions will be isolated and examined for possible differences in the nucleoproteins of tissues of treated and control animals.

Progress: This project is being initiated at the present time.

Significance for Cancer Research (NCP Objective 3, Approach 2)

Level of Effort: Professional Man-Years: 1 month

Serial No. NCI-330

1. Laboratory of Biochemistry
2. Cytochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The development of nuclear magnetic resonance (NMR) methods for detecting foci of cancer cells and following their course of growth

Previous Serial No.: Same (plus 1965, NCI-332)

Principal Investigators: Dean Burk and Mark Woods

Other Investigators:

Cooperating Units: L. R. Maxwell, and Lloyd Kaplan, Naval Ordnance Lab.; I. D. Weisman and Lawrence H. Bennett, National Bureau of Standards; Colin Chignell, LCP, NHLI

Man Years:

Total: 1

Professional: 1

Other: 0

Project Description:

Objectives and Methods Employed: NMR, first discovered in 1945 by Purcell and Bloch, has now become well established as a technique in analytical chemistry, and solid-state physics, and in this project has now been used for the first time to detect cancer in animals. Materials are studied by utilizing the interaction of the magnetic moment of atomic nuclei, in this instance protons, mainly of water. The nuclear magnetic moments behave like weak bar magnets. When the atoms are placed in a uniform external magnetic field, the nuclear magnetic moments align themselves parallel to this external field. The moments can be rotated away from the uniform magnetic field direction by applying radio-frequency (rf) energy at the resonance frequency. When the rf energy source is removed, the nuclei return to their positions parallel to the magnetic field in a characteristic, measurable time, known as the spin-lattice relaxation time T_1 . The duration of T_1 is influenced by the surroundings of the atom and the motions of other atoms. Changes in T_1 reflect changes in the atomic surroundings.

Major Findings: In these studies, measurements of T_1 were made at ambient temperature using a conventional pulsed NMR spectrometer. A mouse's tail, with or without tumor growing on it, was placed in a wire coil, which formed the spectrometer probe, for rf irradiation and signal detection. Measurements of T_1 , taken as a function of time, and in the presence and absence of tumor on the tail, revealed a different value in the presence of the tumor, namely, about 0.6-0.8 seconds compared to 0.3 seconds in the absence of the tumor.

Publication No. 1 listed below, received, as a result of NBS press release, remarkably widespread press coverage, and resulted in over 300 reprint requests, in spite of this being the day and age of xeroxing. Many scientists have contacted the authors of the article wanting to extend its possibilities to a variety of systems and by a variety of methods, as very briefly indicated under "Cooperating Units" above.

Significance to Cancer Research: By using magnets with larger sample space, experiments could be performed on larger animals and even humans, after development of proper coils of differing designs adapted for fitting different parts of the host body. If this technique can be developed and applied at a practical level, competent technicians should be able to test a patient in a matter of minutes, so that perhaps NMR could take a place along side of thermography and radiography as a nonsurgical technique for detecting and monitoring cancer growths.

Honors and Awards: Elected Knight of Mark Twain (Mark Twain Society), in succession to the late Sir Alexander Fleming; Elected Foreign Scientific Member, Max-Planck Institute for Biochemistry, Munich, Germany.

Publications:

1. Weisman, I. D., Bennett, L. H., Maxwell, L. R., Woods, M., and Burk, D.: Recognition of cancer in vivo by nuclear magnetic resonance. Science 178: 1288-1290, 1972.
2. Ardenne von, M.: Selective multiphase cancer therapy: Conceptual aspects and experimental basis (Translation by D. Burk). Adv. Pharmacol. Chemother. 10: 339-380, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-330

Title: The development of nuclear magnetic resonance (NMR) methods for detecting foci of cancer cells in animals and man.

Principal Investigators: Dean Burk and Mark Woods

Section: Cytochemistry Section

Lab/Branch: Laboratory of Biochemistry

Goal: To develop nuclear magnetic resonance (NMR) methods for detecting foci of cancer cells in animals and man.

Approach: NMR, first discovered in 1945 by Purcell and Bloch, has now become well-established as a technique in analytical chemistry, and solid-state physics, and in this project has now been used for the first time in vivo detect cancer in animals. Materials are studied by utilizing the interaction of the magnetic moment of atomic nuclei, in this instance protons, mainly of water. The nuclear magnetic moments behave like weak bar magnets. When the atoms are placed in a uniform external magnetic field, the nuclear magnetic moments align themselves parallel to this external field. The moments can be rotated away from the uniform magnetic field direction by applying radio-frequency (rf) energy at the resonance frequency. When the rf energy source is removed, the nuclei return to their positions parallel to the magnetic field in a characteristic, measurable time, known as the spin-lattice relaxation time T_1 . The duration of T_1 is influenced by the surroundings of the atom and the motions of other atoms. Changes in T_1 reflect changes in the atomic surroundings.

Progress: In these studies, measurements of T_1 were made at ambient temperature using a conventional pulsed NMR spectrometer. A mouse's tail, with or without tumor growing on it, was placed in a wire coil, which formed the spectrometer probe, for rf irradiation and signal detection. Measurements of T_1 , taken as a function of time, and in the presence and absence of tumor on the tail, revealed a different value in the presence of the tumor, namely, about 0.6-0.8 seconds compared to 0.3 seconds in the absence of the tumor.

Significance for Cancer Research (NCP Objective 4 Approach 1) By using magnets with larger sample space, experiments could be performed on larger animals and even humans, after development of proper coils of differing designs adapted for fitting different parts of the host body. If this technique can be developed and applied at a practical level, competent technicians should be able to test a patient in a matter of minutes, so that perhaps NMR

INTRAMURAL RESEARCH SUMMARY CONTINUED

could take a place along the side of thermography and radiography as a nonsurgical technique for detecting and monitoring cancer growths.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-334

1. Laboratory of Biochemistry
2. Cytochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The occurrence and function of a stress-modifiable insulin: anti-insulin hormonal complex regulating glucolysis in cancer and normal cells, and its relation to chemotherapy at normal and hyperthermic temperatures

Previous Serial No.: Same

Principal Investigators: Mark W. Woods and Dean Burk

Other Investigators: None

Cooperating Units: Mr. George Vlahakis, Laboratory of Biology, DCBD, NCI; and Dr. Walter E. Heston, Chief, Laboratory of Biology, DCBD, NCI

Man Years:

Total: 3 3/4

Professional: 3

Other: 3/4

Project Description:

Objectives: To determine the extent to which hormonal regulation (insulin: anti-insulin) of glucolysis functions as a growth and cell function control system in different cancers and normal cells, and to find ways in which such systems can be manipulated as an adjunct to in vivo therapies (e.g., chemotherapy and stimulation of host defensive cells (RES). This project most closely supports the National Cancer Plan in area 6-THERAPY (i.e., "to maintain maximum control of the cancerous process in patients not cured" through modification of control mechanisms of cell metabolism (objective 6, approach 10).

Methods Employed: The glycolytic properties of tumors and normal tissues at various glucose levels are determined in the presence and absence of inhibitors such as 2-deoxy glucose, steroids, compounds with steroid-like anti-glycolytic action such as delta¹-testololactone, diethylstilbestrol, Podophyllum derivatives, and substances which counteract such glycolytic inhibition (e.g., insulin and bacterial endotoxins). We used these techniques in further studies of the glycolytic properties of a large number of primary and transplanted liver tumors and primary and transplanted mammary tumors of mice.

Major Findings: A basic question is whether in the origination of cancer there is an obligatory increase in glucolytic capacity of the transformed cells

relative to the normal cells of origin. An essential connection between hormonal regulation (insulin:anti-insulin) of glycolysis and cancer growth in vivo has been indicated by work with various normal and malignant mammalian cell systems. The accumulated data support the view that development of even the most slow growing and least deviated cancers involves significant increases in the glycolytic capacities of the cells.

Spontaneous mouse hepatomas almost always had increased anaerobic glycolytic capacities relative to host or normal livers. However, certain apparent "exceptions" were encountered (project NCI-334, 1971-'72) where large non-necrotic primary hepatomas in 21 month old male C3HfB mice glycolyzed in vitro at about the same rates as liver. Since such tumors could have constituted valid exceptions to the rule of glycolytic increase in cancer, further studies were carried out in 7 C3HfB males 18 to 21 months old. Data from 17 primary hepatomas (7 showing "normalization"), and from tumor-free areas in each of the 7 host livers showed that the "normalization" phenomenon is an age-related, glucose concentration-dependent, reversible, inhibition of glycolysis. The data do not indicate the occurrence of primary hepatomas with intrinsically normal liver cell glycolysis. The observed low glycolytic rates in "normalized" tumors probably resulted from tumor-induced stress in the host animals.

Glycolytic determinations in various mouse mammary tumors were continued in strains C3H, C3HfB, C3H-A^{vy}fB, BALB/c, and C57BL mice. In many instances in older (10-18 months) females of C3H strains both primary hepatomas and mammary tumors grew simultaneously in the same individual host animal. In vitro comparisons of the glycolytic properties of such tumors, as well as of both types of cancer from different individual mice showed certain marked differences in the glycolytic properties of the two types of tumor. For example, maximum rates of glycolysis occurred at about 0.2% glucose in all primary mammary tumors ($Q_{CO_2}^{N_2}$ - 8 to 14) whereas in the hepatomas maximum rates occurred only at much higher levels of glucose. With 2.7% glucose in primary hepatomas the $Q_{CO_2}^{N_2}$ was about 3.0 and in transplanted hepatomas about 5 to 7. At 0.27% glucose the rates of glycolysis in hepatomas were much lower ($Q_{CO_2}^{N_2}$ in primary hepatomas about 1.0, and in TP hepatomas 1.0 to 3.0). The one cholangioma studied (TP 16654) had an average Q glycolysis of 6.0 at 0.27% glucose and 7.5 at 2.7% glucose.

Glycolysis in primary mammary tumors in vitro was inhibited 19 to 40% ($P < 0.01$ to 0.025) by anti-insulins (testosterone or delta 1-testololactone), and there were small (7 to 15%) but significant ($P < 0.01$ to 0.025) stimulations by exogenously added insulin. When 2.7% glucose was replaced by 2.7% fructose in mammary tumors the anaerobic acid production (fructolysis) was 80% lower than with glucose. The greatly increased K_m with fructose apparently involves an intensification of the anti-insulin effect at the hexokinase level. This is true for both endogenous and exogenously added anti-insulins. Correspondingly, insulin effects with fructose, or with glucose, depends on the levels of insulin and anti-insulin factors.

There is considerable evidence that the insulin:anti-insulin system can act as a growth-controlling mechanism in various mammalian tissues. While the anabolic effects of insulin (e.g., stimulation of protein synthesis, increases in DNA polymerase and DNA synthesis) may not be measureable until several hours (4 more or less) after insulin addition the first effects on glycolysis are almost immediate (5 to 15 minutes). The present studies indicate that in freshly harvested primary mammary tumors or hepatomas anaerobic glycolysis is to a

variable extent responsive to exogenously added insulin and anti-insulins. Inter-tumor differences in susceptibility to this type of regulation occur, often between different tumors from the same host animal. It is suggested that insulin may at first act immediately to stimulate the glucokinase reaction in cellular compartments where glucolytic phosphorylation (or later products formed there from) play a critical role in the control of cancer growth. Thus, insulin may "induce" the synthesis of cellular DNA and proteins, including more hexokinase itself.

Certain transplanted mammary tumors (e.g., C57BL/Moore) with high glucolytic potential $Q_{CO_2}^{N_2}$ 10 to 15) seem to have undergone a considerable loss in sensitivity to insulin:anti-insulin regulation. Such shifts in the cell potential for anaerobic glucolysis have been seen in various tumor systems studied in this project. The change seems to be always towards more glucolytic activity with lessened sensitivity to anti-insulin inhibition of glucolysis and lessened stimulation by insulin, i.e., the cells become refractory to this type of host modulated metabolic regulation. Such cells have shown increased growth capacities (decreased induction period following transplantation, increased in vivo rates of growth, decreased susceptibility to inhibition by a state of host stress (increases in anti-glucolytic action)).

Significance to Cancer Research: From studies in diverse types of normal tissues (kidney, brain, spleen, liver, leukocytes, etc.) and different types of tumors (melanomas, mammary carcinomas, tissue culture-originated sarcomas, lymphocytic and granulocytic leukemias, hepatomas, cholangioma, reticulum cell neoplasms, etc.) it is clear that essentially the same basic type of steroid-insulin control of glucolysis occurs. Where the relevant non-malignant cells of origin have been available for comparison, the derived cancer cells have shown not only increased rates of anaerobic glucolysis, but probably more important, decreases in susceptibility to steroid-induced inhibition of glucolysis. Similar progressive decreases in susceptibility to such glucolytic regulation were found in tumor spectra based on progressive anaplasia (e.g., S91 melanoma derivatives and Sanford tissue culture tumor clones). There is considerable evidence that glucolytic capacity also plays a crucial role in the functioning of defensive cells of the RES. Successful chemotherapy may depend upon knowledgeable exploitation of differences in glucolytic regulation in cancer and in the normal defensive cells (RES).

Proposed Course of Research: It is recommended that the lines of investigation presently underway be maintained if the project is to be continued.

Honors and Awards: None

Publications:

1. Woods, M. W., and Vlahakis, G.: Anaerobic glycolysis in spontaneous and transplanted liver tumors of mice. J. Nat. Cancer Inst. In press

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-334

Title: The occurrence and function of a stress-modifiable insulin:anti-insulin hormone complex regulating glycolysis in cancer and normal cells, and its relation to chemotherapy at normal and hyperthermic temperatures

Principal Investigators: Mark W. Woods and Dean Burk

Section: Cytochemistry Section

Lab/Branch: Laboratory of Biochemistry

Goal: To help in achieving in vivo control of tumor growth and to augment other types of therapy (e.g., immunotherapy, chemotherapy, hyperthermia).

Approach: Determine the nature and extent to which insulin:anti-insulin hormonal regulation of glycolysis functions as a metabolic control mechanism in cancer and normal mammalian cells, and discover ways in which the system can be manipulated as an adjunct to in vivo therapies (e.g., chemotherapy and hyperthermia) including the role of such glycolytic regulation in host defensive cells (RES).

Progress: From studies in diverse types of normal tissues and different types of tumors, it is clear that essentially the same basic types of steroid-insulin control of glycolysis occurs in all tissues. Where the relevant non-malignant cells of origin have been available for comparison the derived cancer cells have shown not only increased rates of anaerobic glycolysis, but probably more important, decreases in susceptibility to steroid-induced inhibition of glycolysis. There is considerable evidence that glycolytic capacity also plays a crucial role in the functioning of defensive cells of the host RES. Successful chemotherapy may depend upon knowledgeable exploitation of differences in glycolytic regulation in cancer and in the normal defensive cells (RES).

Significance for Cancer Research (NCP Objective 4 Approach 1): These studies have a particular bearing on the problem of control of tumor growth (NCP objective 4). The data indicate that insulin:anti-insulin regulation of glycolysis functions as a growth controlling mechanism that becomes more or less nonfunctional in cancer. The same type of control system apparently operates in normal defensive cells (lymphocytes, plasma cells) so that agents which may retard cancer cell growth over glycolytic inhibition may also retard the beneficial effects of glucose metabolism in defensive cells. Fortunately, it appears that at least some tumors are less homeostatic with respect to certain inhibitors than is the case with normal tissues. More should be learned about possible potentiators of the anti-glycolytic agents.

Level of Effort: Professional Man-Years: 3

PHS-NIH
Summary Report
July 1, 1972 through June 30, 1973
Laboratory of Biology
NCI

During the past year significant advances have been made in the four principal areas of research of the Laboratory: 1) Tissue culture techniques, 2) In vitro carcinogenesis, 3) Mammary tumorigenesis in the mouse, and 4) Biochemical genetics in relation to tumorigenesis.

Tissue Culture Techniques: The principal efforts of Dr. Evans and Dr. William Taylor have been in this area. They have further designed and tested their pulsatile perfusion system for cell culture, and a new commercial device with a plastic coil for cell attachment for growing large masses of cells, and Taylor has developed a media with an autoclavable serum-substitute that can be used to grow significant numbers of certain cell lines without sacrificing their general biological characteristics or susceptibility to clinically important viruses. All of these are great advances in the culture of particularly nonmalignant cells in large amounts. They will be of great value for the biochemist, for growing large masses of cells for vaccine production, or for mass cultures for production of hormone-like substances or other biologically active components not yet synthesized in the laboratory.

In Vitro Carcinogenesis: Principal interests and contributions of Dr. Sanford, Dr. Gantt, Dr. Tucker, and Dr. Longfellow (Guest Worker) fall in this area.

Dr. Sanford has made further observations of characteristics of cells associated with the neoplastic transformation. Some of these are cytologic changes, loss of fetal antigens of cultured fetal cells undergoing neoplastic transformation, dynamic morphology and surface membrane changes of the cells, and immunologic discrimination between neoplastic and nonneoplastic cells in vitro. Preliminary studies of Dr. Tucker indicate that mouse peritoneal macrophages activated by endotoxin can selectively kill neoplastic cells in culture.

Further observations have been made relative to the causes of spontaneous neoplastic transformation of cells in vitro. Lack of correlation between presence of gs antigens of endogenous murine leukemia virus and neoplastic transformation suggest that the transformation is not caused by this virus. In work with Evans and Gantt, low molecular weight fraction of horse serum has been implicated in accelerating, and fetal bovine serum components in inhibiting neoplastic transformation.

Reducing oxygen level increases growth of cells. Sanford and Parshad tested lines of fibroblasts from people with genetic disorders predisposing to cancer for carcinogenesis with benzo(a)pyrene. However, the cells showed no evidence of malignant transformation. They became heteroploid but did not establish continuous cell lines characteristic of malignancy.

Dr. Gantt's interests are primarily in nucleic acid methylation and the possibility that an aberrant methylation is a fundamental cause of the neoplastic transformation. A signal contribution has been his development of an assay 100-fold more sensitive which is quantitative and specific for the methylated bases. He observed a difference in methylase activity between neoplastic and nonneoplastic cell lines in culture. Longfellow and Gantt are making further comparison of these paired neoplastic and nonneoplastic cell lines isolating a DNA replication complex and characterizing associated DNA methylase and DNA polymerase activities to see if there are differences associated with neoplasia. Dr. Smith and Dr. Gantt are extending the observations on methylase to oncogenic viruses. C-type sarcoma viruses have two methylase activities while leukemogenic viruses also C-type viruses have only one. B-type virion of mammary tumors has no detectable RNA methylase activity, but a methylase is found in intracytoplasmic A particles thought to be the immature form of the B particle. Demonstration of a definite methylase aberration in the neoplastic process would offer a point of attack for chemotherapy.

Mammary Tumorigenesis in the Mouse. Work in this area has been concerned chiefly with the mammary tumor virus itself and the genetic control over its transmission and replication. The latter is also covered by contract with the Netherlands Cancer Institute.

Dr. Smith has been working with the intracytoplasmic A particle which is thought to be the immature form of the B particle, the mammary tumor virus, but he has found that purified intracytoplasmic A particles are noninfectious in contrast to B particles. In immunologic comparison of purified A and B particles he has found cross-reacting antigens. He has isolated and characterized a high molecular weight DNA associated with the A particle which may be associated with a reverse transcriptase for replication. Comparison of these observations with the B particle should help to elucidate the replicative cycle of the mammary tumor virus.

Mr. Vlahakis and Dr. Heston have been concerned with the various lines of the mammary tumor virus such as MTV, NIV, etc., that differ in their capacity to induce tumors and in their transmission. They have shown that even different lines of MTV differ in tumor inducing capacity depending upon their mouse strain of origin. They are also very much interested in NIV, the line of mammary tumor virus that appears to be

transmitted genetically as a provirus. With the use of a mouse strain in which they have shown that 90 percent of the females get mammary tumors due to NIV, it is hoped that a genetic analysis of the transmission of this postulated provirus can be made including location of it on the chromosome with linkage tests.

Dr. Hoffman is developing immunologic tests for the NIV antigen to enable such a genetic analysis to be made. He is also working with Dr. Hilgers of the Netherlands Cancer Institute in carrying out some of the tests for linkage between the provirus and a number of biochemical markers. Genetic transmission of NIV is of particular importance because if there is a breast cancer virus in women it is probably transmitted in a similar fashion.

Using avian myeloblastosis virus, Dr. Stromberg has developed techniques for separating the core from the whole virion and further separation of the nucleoid from the core and then characterizing each component immunologically and biochemically. This year he has been working in Dr. Spiegelman's laboratory where he has been concerned with the mammary tumor virus and identification of a possible human breast cancer virus by the identification of a reverse transcriptase. He is working with secretions from the non-lactating breast and applying his techniques for separating components of the virion. He is convinced that we must work with these purified viral components if we are to demonstrate a mammary tumor virus in women.

Biochemical Genetics in Relation to Tumorigenesis. Dr. Hoffman and Dr. Grieshaber have been interested in chemical and genetic interaction in expression of protein function in neoplasms. Basic genetic work has been done on the catalase system, identification of the gene, linkage, and development of congenic strains. Dr. Grieshaber has worked out a method for isolating RBC catalase so complete chemical and physical analysis can be carried out. In a C3H-A^{VY} hepatoma catalase activity was 40 to 60% lower than in the normal tissue offering a system for study of enzyme and protein regulation in neoplastic tissue.

Other Investigations. Results from testing the antifertility drug Enovid for carcinogenicity in mice are now in press. The results suggest that the drug is relatively safe in respect to carcinogenicity in women at the low dose level that will prevent fertility. However, some attention should be given to possible induction of pituitary tumors in older women who have taken the "pill" in earlier life.

Serial No. NCI-3208

1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Inheritance of the Expression of MTV Group Specific (gs)
Antigens in Inbred Mice

Principal Investigator: Harold A. Hoffman

Other Investigators: Charles K. Grieshaber
Wendell Niemann (New York University Medical School)
Warren Evans (Laboratory of Biochemistry, NCI)
Edward Boyse (Sloan-Kettering Institute)

Man Years:

Total:	5
Professional:	2
Other:	3

Project Description: Objective 3 Approach 3

Objectives: To study by methods appropriate to biochemical and quantitative genetics the inheritance of proteins in the mouse and other mammals and to determine the genetic and non-genetic relationships between the proteins and tumors. To determine the primary structure of the normal protein and the aberrant or mutant protein in order to define the gene or genes controlling their production and to determine the time of gene action during the process of neoplastic transformation.

Methods: In these investigations mice from 18 inbred strains and sub-strains, which represent at least 6 different independent lines of known genetic origin, are used as sources of proteins and tumors, and for the production of isologous antisera. New Zealand white rabbits of known immunoglobulin type are used for the production of heterologous antisera, and non-human primates of the order anthropoid are used as a source of protein.

Immunochemical methods, agar-gel-double diffusion, immunoelectrophoresis, and immunofluorescent absorption are used in the detection and characterization of different proteins. Physical and chemical techniques of starch-gel electrophoresis, agar-gel electrophoresis, polyacrylamide-gel electrophoresis, and chromatography, both gel

filtration and ion exchange, are used to isolate and classify different proteins. The fine structure and minute differences between closely related proteins are determined by peptide map analysis (fingerprints) and amino acid analysis.

Inheritance of tissue and erythrocyte catalase in inbred mice:

Testing of liver catalase activity in the Inbred Recombinant (IR) lines of Dr. Donald Bailey (The Jackson Laboratory) indicated a possible loose linkage between the low liver catalase locus, Ce-1, and autosomal glucose-6-phosphate dehydrogenase, Gpd-1, on chromosome 4 (Linkage group VIII). Backcross segregants from the cross (B6 x C)_F₁ x C57BL/6 are being tested for the loci Mup-1, Gpd-1 and Ce-1 in a three-point linkage analysis.

Genetic analysis of the erythrocyte catalase locus, Cs-1, (chromosome 2, linkage group V) has established a multiple allelic system with at least three alleles. The three type strains are BALB/c (Cs-1a), C57BL (Cs-1g), and NBL (Cs-1h). (Feinstein has designated his acatalasemic and hypocatalasemic mutants with alleles b-f.)

Production of three congenic acatalasemic strains on BALB/c, C57BL, and C57BL/6 backgrounds: in order to study the biochemistry of the low liver catalase gene and the acatalasemic gene in two different inbred strains of mice, the inbred strains must be congenic with respect to one of the two loci in question. Therefore, experiments were initiated to place the acatalasemic gene on three different inbred backgrounds where the three inbred strains differed for the low liver catalase genes. The sixth backcross generation has been completed, which means that the acatalasemic gene theoretically is on a 99.2% inbred background. Three more backcross generations are to be completed at which time the acatalasemic gene theoretically will be on 99.9% inbred background.

Erythrocyte catalase was purified from BALB/c and C57BL/6 inbred strains by Sephadex G-100 gel filtration and chromatography on DEAE cellulose. Three peaks of active catalase were found which correspond well to those seen in RBC's from other mammalian sources. With our method, catalase can be isolated (350 fold purification) from small quantities of packed erythrocytes in less than two days, enabling us to biochemically and immunochemically characterize the enzyme molecule in a state where interaction of cytoplasmic control factors are absent.

Inheritance of the expression of MTV group specific (gs) antigens:

The highly susceptible C3H-AVYfB inbred strain and the resistant C57BL strain are being used for the genetic analysis of the susceptibility to MTV and subsequent appearance of mammary tumors. The expression of the viral antigen--either MTV-gs or NIV-gs--is detected by the immunofluorescent absorption (IFA) test. The goal of the experiment

is to locate in the mouse genome the genetic site for gs expression (the provirus) and to correlate the segregation of viral antigen specificity and appearance of mammary tumors. The anti-MTV sera, which was made against the B-particle band from C3H milk, has an antibody titer of 1/128 after absorption with C57BL/6 milk. IFA testing of primary C3H, GR and C3H-A^{VY}fB mammary tumors showed the presence of viral antigen in these tissues, while IFA tests of a transplanted mammary tumor from C57BL/He (generation 29) and a primary mammary tumor from BALB/c gave negative results. It appears that the anti-MTV sera is highly specific for MTV-gs and will detect NIV-gs only when it is in very high concentration.

Proposed Course of Project: Isolation of murine kidney catalase will be carried out using a slight modification of the procedure described for erythrocyte catalase. Since there are differences in the electrophoretic mobility of kidney catalase in tissue homogenates, a purified preparation of enzyme from the kidney is essential to genetic characterization of catalase.

Studies on the correlation between the inheritance of erythrocyte and liver catalase and granulocyte catalase and peroxidase will be continued using the congeneric strains for the acatalasemic gene.

Isolation of B-particles from primary C3H-A^{VY}fB mammary tumors and production of a specific anti-NIV-gs sera are the primary goal for the next phase of the study. Production of first generation backcross females has been initiated; these females will be tested for the presence or absence of viral antigen and for biochemical markers on nine different chromosomes.

Publications:

Acton, R., Blankenhorn, E., Douglas, T., Owen, R., Hilgers, J., Hoffman, H., and Boyse, E.: Variations among sublines of inbred AKR mice. Nature, in press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3208

Title: Inheritance of the Expression of MTV Group Specific (gs) Antigens in Inbred Mice

Principal Investigator: Harold A. Hoffman and Charles K. Grieshaber

Section: General Biology

Lab/Branch: Laboratory of Biology

Goal: Genetic mapping of the locus/loci controlling the susceptibility to mammary cancer

Approach: This study utilizes the techniques of quantitative genetics, biochemistry, and immunochemistry. Female first generation backcross mice are tested for the presence or absence of viral group specific antigen by immunofluorescent absorption and for the allelic distribution of biochemical marker genes on nine different chromosomes and morphological characters on three different chromosomes. The data are analyzed by standard genetic linkage programs.

Progress: In the first 9 months of the study, specific absorption of the rabbit anti-MTV sera, titering of the sera with the target cell test system, and characterization of the allelic distribution of the biochemical markers were completed.

Significance for Cancer Research (NCP Objective 3 Approach 3)

This study provides an animal model for the determination of genetic and non-genetic factors for human breast cancer.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-3211 SAME
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies, by tissue culture and cell culture methods and by amplifying methods, of the malignant cell and of the normal cell from which it arises; studies on the mechanism of transformation of the normal cell into the malignant cell; studies on the characterization and identification of such cell; studies on associated significant subjects.

Principal Investigators: V. J. Evans, K. K. Sanford, R. R. Gantt, W. G. Taylor, F. M. Price, R. W. Tucker (Research Associate), M. L. Taylor (Guest Worker), and C. H. Fox (Guest Worker).

Other Investigators: See individual project reports Nos. 3212, 3213, 3234A, 3257, 3262, 3263, and 3264.

Man Years: July 1, 1971 through June 30, 1972

Total	19-3/4
Professional	11
Other	8-3/4

Project Description: See Project Title above.

Outline of Activities:

The long-term objectives of the personnel of the Section are indicated in the general title above and are described in detail in the individual projects. To carry out the objectives considerable time has been spent in the past years and will continue to be spent as required in order to maintain and develop superior technology and instrumentation, to advance current problems, particularly those related to the National Cancer Plan's 7 objectives, and to anticipate maintaining eminence in the primary interests.

Some of the unusually time consuming details related to evaluating equipment and supplies for long-term large-scale antibiotic-free tissue culture work are mentioned because they have resulted in recently designed equipment and quality control supplies that others outside the laboratory have found useful: such things as unique glassware washing equipment and all-glass water distillation units. Stainless steel autoclaves operated with a special steam generator and heat exchanges for distilled water steam have been tested and work effectively. Two precise electronically controlled constant temperature rooms have been designed, constructed, and tested; these have been copied both within and outside the NIH. The primary source water distillation unit is supplying adequate volumes of distilled water for washing and to supply a unique triple glass distillation unit for preparing protein-free medium for our own and others' use.

Currently still under need of correction are the so-called laminar flow clean hoods used for renewal of nutrient fluids on cell cultures. These were purchased from lowest bidder several years ago prior to completion of Building 37. Subsequently, new more satisfactory types have been designed. The ones currently in use have been found to be defective in the following respects: They build up high heat intensity in areas to 110°F and the air conditioning engineers to date have found no way to dissipate this. The perforated design table in these boxes is such as to make it difficult to keep clean or to clean the boxes, and more important from the workers' point of view, the present hoods are not safe for handling biologically hazardous material. There are additional other defects, all related to potentially contaminating cultures. Considerable time has been devoted to exploring substitutes for these laminar flow tissue culture hoods that would be both biohazard hoods for investigators' safety as well as effective for the satisfactory culture of cells, tissues, and organs in vitro. Boxes currently available and satisfactory for biohazard work are still cumbersome for use in modern tissue culture work and many tend to trap heat, as noted above, build it up around cultures, and expel it into operator's face. The rigid standards currently used by NIH are blocking the opportunity for individual labs to test products and find ones safe and suitable for non-biohazard work. The present standards are based only on needs for virologists and seriously handicap cell biologists' needs and the opportunity for them to run surveys and on-site tests on newly developing equipment.

The microcinematograph and special unique film to be used to analyze in more detail dynamic morphology and growth characteristics of living non-malignant and malignant cells under diverse culture conditions is gradually coming into productive use. The temperature control system for the cultures has recently been calibrated for maximum efficiency.

The homeostatic perfusion culture system alluded to last year is fairly well under way and details are to be found in Serial No. NCI-3262. New mass cell growth devices incorporating perfusion systems designed in the laboratory are in recent test, as described in Serial No. NCI-3263.

A compact lab bench type liquid nitrogen freezer for preservation of cell lines, steel lined for safety, and with a custom designed inventory system, has been installed and has been tested, found to be safe and efficiency, and is beginning to have wide use in the field of cell preservation because of its safety features and flexibility of use and compactness for crowded laboratories.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3211

Title: Studies, by tissue culture and cell culture methods and amplifying methods, of malignant cell and normal cell from which it arises; mechanism of transformation of normal cell into malignant cell; characterization and identification of such cells; associated significant subjects.

Principal Investigator: V.J.Evans, K.K.Sanford, R.R.Gantt, W.G.Taylor, R.W.Tucker

Section: Tissue Culture Section

Lab/Branch: Laboratory of Biology

Goal: To maintain facilities for carrying out objectives of studying in cell culture and related disciplines the differences between normal and malignant cells.

Approach Facilities are constantly monitored to maintain quality work in tissue culture so that investigators in the laboratory are enabled to carry out their work. Included in these facilities are 1) Unique high efficiency constant temperature incubator rooms with no more than 0.2°C variation maximum; 2) mechanical stainless steel glass washing equipment requiring high quality distilled water and special washing compounds; 3) Specialized custom designed glassware; 4) Custom designed triple glass distillation units for preparing chemically defined media (CDM) to be used in absence of serum proteins; 5) Custom designed liquid nitrogen freezers for storage of cells; 6) Distilled water and stainless steel steam autoclaves for equipment to be used with cells in CDM; 7) Selection and testing of quality chemicals for CDM; 8) Custom designed cinephotomicrographic equipment; 9) Laminar flow clean benches and rooms for all culture work; 10) Perfusion devices for cell culture; 11) Selection, testing of various exotic serums.

Progress: Progress is defined in the quality of experimental work described in serial numbers NCI-3212, 3213, 3234, 3257, 3262, 3263, and 3264.

Further significance of the value of our custom laboratory equipment and supplies is the frequency with which we are consulted by other tissue culture laboratories being set up or altered. Still of great concern are our laminar flow containment cabinets, which have an exceptional record for sterility but are still not satisfactory because of the amount of heat produced and not removed. These have taken much time to try to obtain some as yet unresolved solution.

Significance for Cancer Research (NCP Objective 2 Approach 4)
and (NCP Objective 3 Approach 2,3)

The activities in this Section could not be carried out unless the physical facilities and certain specialized equipment are obtained and kept functioning.

Level of Effort: Professional Man-Years: 1/4

Serial No. NCI-3212, Project 1 SAME

1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project 1, Title: Studies of cell nutrition and metabolism in vitro.

Principal Investigators: V.J.Evans and F.M.Price

Other Investigators: K.K.Sanford, R.R.Gantt, W.G.Taylor, R.F.Camaller,
R.A.Dworkin

Man Years: See Serial No. NCI-3211

Cooperating Units: None

Project Description: National Cancer Plan 2.4, specifically 2.4.3.3
and 2.4.3.8 and 3.2

A program to determine the nutritional and physico-chemical needs of cells in vitro for better utilization of such cells in a variety of problems. Primarily the cells are for the study of the phenomenon of spontaneous malignant transformation in vitro. The program also contributes to advancing tissue culture technology and to supplying basic contributory knowledge for biologic research materials, specifically mammalian cells, including human cells.

Objectives:

1. To improve tissue culture technology so that more cell types including human cells can be cultured for long-term in protein-free or protein-supplemented media without experiencing the usual degenerative changes.
2. To improve media, cultivation techniques, and separation methodology for large-scale production of normal and malignant human and mammalian cells.
3. To improve culture conditions of cells so that rigidly controlled cells can be used to study the differences between nonneoplastic and malignant cells at the nutritional, metabolic, and morphologic levels.

Methods Employed: Freshly explanted mouse cells, long-term mouse cells, and long-term human diploid or quasi-diploid cells were grown in media of varying formulations with or without protein supplement. Substances used as supplements were: human α 1 protein; bovine serum albumin; large and small molecule fractions of mare and stallion horse serum and fetal bovine serum; mare and stallion serum; fetal bovine serum; and Witte's peptone.

Major Findings:

1. Development of a chemically defined medium for growth of freshly explanted and long-term lines.

- a) Growth of primary cultures: Attempts have been made to improve the

formulation of our culture medium in order to provide a medium which will support growth of primary cultures without the use of serum or protein hydrolysates. At the same time we were trying to eliminate unnecessary ingredients. Major changes thus far have been: 1) to change the buffering capacity of the medium to prevent an alkaline pH by decreasing the amount of bicarbonate and adding disodium phosphate; 2) to increase the concentration of glucose, several amino acids (particularly L-alanine and L-glutamine) and the B vitamins; 3) the addition of putrescine and sodium pyruvate; 4) to delete from the formulation 3 amino acids, 6 coenzymes and 3 nucleic acid precursors.

Results thus far have been encouraging, although no established or long-term cell lines have been obtained. Our best improved medium to date has supported growth of primary C3H mouse embryo cultures for 9 culture generations over a period of approximately 15 weeks. This compares favorably with the chemically defined medium NCTC 135, which we have been using, which has supported growth of primary mouse embryo cultures for 2 or 3 culture generations before they finally died out, or lived indefinitely, but only with assiduous efforts, usually employment of strong adaptation pressures predicated upon constant observation. It appears from our results so far that freshly explanted cells can grow in completely defined medium, but a high percent of the cells are more fragile upon subculturing so that there is an ultimate decrease of the cell population below minimum inoculum numbers.

b) Growth of established cell lines: A new formulation of medium has been prepared and used on 4 long-term cell lines growing in completely chemically defined medium NCTC 135 to determine if it would support a larger population of these cell lines than medium NCTC 135. The four lines used represented 4 species of mammalian cells, human, monkey, Chinese hamster, and C3H mouse. All four cell lines could be grown as long-term cultures in medium NCTC 163, although the monkey cells did not grow so rapidly as the other lines. In a quantitative study comparing growth rates of these cell lines in medium NCTC 135 and in medium NCTC 163 over a period of 7 days, all but the monkey cells reached a higher population in medium NCTC 163 than in NCTC 135.

2. Growth of freshly explanted cells in medium supplemented with human ~~and~~ 1 protein, bovine serum albumin, and/or Witte's peptone.

Initially all studies were carried out with NCTC 135 as the basal medium. These studies are reported in Serial No. NCI-3257. Later studies were made with peptones or peptone dialysates in the basal medium NCTC 163. A 15% Witte peptone supplement to NCTC 163 has been quite successful as a supplement. Two cell lines from mouse embryos were initiated in NCTC 163 supplemented with Witte's peptone. Both of the cell lines grew fairly well at first and then went into a growth lag at about 7 culture generations. This type of growth lag is common to freshly explanted cells; the cell lines recovered after several weeks and now have been cultivated, with heavy growth for about one year in this medium.

A bacto-peptone dialysate was also used as a supplement to NCTC 163. Two cell lines were grown in this medium without success. Apparently the bacto-

peptone dialysates can only be used as a supplement to already established long-term cell lines, as has been demonstrated by other investigators in other laboratories.

3. The influence of low oxygen in establishing and maintaining cells in vitro.

a) Freshly explanted mouse cells: There is evidence that cells grown in an atmosphere of low oxygen tend to show less chromosome damage than those grown in an atmosphere of CO₂ in air. There is also evidence that cells grown in lower amounts of oxygen tend to clone better. We attempted to determine whether cells initiated and maintained in an atmosphere of low oxygen can be established and maintained as normal diploid cells and/or can be prevented from becoming malignant. To date 12 cell lines were initiated in an atmosphere of 1% oxygen, 10% CO₂, and 89% nitrogen. Half of the cell lines were grown in medium NCTC 163 and half in Dulbecco-Vogt medium. All cell cultures in NCTC 163 grew poorly in the 1% oxygen and never became established. Those cultures growing in Dulbecco-Vogt medium in 1% oxygen grew as well or better than sister cultures in an atmosphere of 10% CO₂ in air. This information indicates that the use of 1% oxygen may require specific media formulations or perhaps specific buffering systems, since in the 1% oxygen atmosphere the pH of medium NCTC 163 remained constantly low. Further studies must be carried out to determine the long-term results of these experiments.

b) Long-term diploid normal human cells: Cell line Wi-38 is widely used in studies on aging, cancer, and vaccine production. Exploratory studies are in progress using this line as a prototype to attempt to resolve why human diploid cells have a finite life and to try to reverse this phenomenon by unique culture and nutrition conditions extrapolated and extended from the above studies. Initial experiments growing Wi-38 cells in an atmosphere of 1% oxygen have been unsuccessful. The use of other media as indicated above may be necessary in order to better grow these Wi-38 cells in an atmosphere of low oxygen. Other studies experimenting with different gases on Wi-38 cells are described in Serial No. NCI-3263.

4. Capacity of C3H mouse embryo cells to grow in medium containing gelding, mare, or stallion serum from a commercial source.

Three pools of C3H mouse embryo cells were explanted to test stallion, mare, and gelding serums. Two of the pools were from C3H/HeN embryos and the third pool from C3H/He embryos. In all 3 pools, growth in NIH gelding horse serum (control serum) was less satisfactory than in commercial gelding, mare, or stallion serum. Of the commercial serums in long-term experiments, the best growth was obtained with stallion serum. The next best growth was obtained with the mare serum, although the difference in growth potential between it and gelding serum was practically non-significant. In stallion serum, irrespective of the size of initial population, all cells have about the same rate of proliferation. The subculture interval was longer in the C3H/He cells than in the 2 pools of C3H/HeN cells in the mare and commercial gelding serums. In these 2 serums the size of initial inoculum appeared to have an influence; the smallest inoculum appeared to grow more slowly. A manuscript describing these results has been published.

5. Growth of C3H₁₀/HeN mouse embryo cells in large and small molecule fractions of stallion and mare serum.

A study has been designed to determine the effects of large and small molecule fractions of stallion and mare serum on the growth and spontaneous neoplastic transformation of C3H mouse embryo cells. The results on transformation are discussed in Serial No. NCI-3212, project 2. Three pools of C3H₁₀/HeN mouse embryo cells were set up and grown in the following media combinations: NCTC 135 + large molecule fetal bovine serum (Lg FBS)(control); NCTC 135 + large molecule fraction of stallion serum (Lg St); 135 + large molecule fraction of mare serum (Lg M); 135 + Lg M + small molecule fraction mare serum (Sm M); 135 + Lg M + small molecule fraction of stallion serum (Sm St); 135 + Lg St + Sm St; 135 + Lg St + Sm M; 135 + Lg FBS + Sm M; 135 + Lg FBS + Sm St.

In all 3 pools the best growth has been obtained by those cells growing in medium containing Lg FBS. However, for one pool of cells the best growth was obtained in medium containing Lg FBS and Sm M, and in another cell pool growth was best in medium containing Lg FBS and Sm St. For all three cell pools, poorest growth was initially found for those cells in medium containing either Lg M or Lg St with no other supplement. There has been a variation in growth among the 3 pools for the other cells grown in media containing either Lg M or Lg St in combination with Sm M or Sm St.

Significance and Proposed Course:

Neither normal (non-malignant) human cells or specific tumor types, whether human or lower animal, are grown as readily in long-term cell culture as are the normal cells of lower animals and fibrosarcomas or highly undifferentiated tumors of lower animals. There are, of course, exceptions to these results. Nutritional factors may have a profound effect on the growth of both normal and malignant cells. The classic example usually cited is the requirement of leukemia cells for asparagine. In order to have a variety of cells for models, both normal and malignant, numerous formulations of chemically defined media, have been evolved. These have varied in amino acids, salines, vitamins, and other components. Some of these have been employed already for their relative capacity to grow two kinds of human tumors, i.e., HeLa and anaplastic squamous cell carcinoma of the nasal septum. Our recently formulated medium NCTC 163 appears to be excellent for the growth of certain cell types and may with other changes grow even more kinds of cells. The continuing program on nutrition and physico-chemical requirements of cells particularly for advancing studies on differences between nonneoplastic and neoplastic cells still relies on: 1) the analytical approach to determine growth promoting factors in known medium supplements such as serum or peptones, and 2) the successful synthetic approach of logical additives to media formulations of chemically characterized compounds. The leads from the serum fractionation studies suggest investigating the importance of hormone-like substances, unknown vitamin-like materials, polypeptides, and perhaps purines, pyrimidines, or their nucleosides. Further simple but time-consuming testing of different ratios of vitamins and amino acids may be rewarding for growth stimulation. It is highly desirable, also, to work with autoclavable media to avoid adventitious agents often found in sera.

For some years it has been held that human diploid cells in culture cannot be maintained indefinitely; they either become heteroploid and continue to proliferate, or they cease to divide after a finite period in vitro, as with Wi-38. For the overall cancer plan, long-term growth of normal diploid human cells is paramount for general studies and mass amounts of cells. Efforts will be and are being made (Serial No. NCI-3263) to develop better media and culture techniques in order to obtain this goal. Studies to determine and isolate specific growth promoters or inhibitors are under way. Experiments involving mass cultures and gas requirements of normal cells are also presently being carried out (Serial No. NCI-3263).

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3212, Project 1

Title: Studies of Cell Nutrition and Metabolism in vitro.

Principal Investigator: V.J.Evans and F.M.Price

Section: Tissue Culture Section

Lab/Branch: Laboratory of Biology

Goal: To determine nutritional and physico-chemical needs of cells in vitro for general use and to establish cell systems for the study of spontaneous malignant transformation in vitro.

Approach Attempts were made to improve the formulation of our chemically defined medium NCTC 135 in order to provide a medium which would support growth of primary cultures without the use of serum or protein hydrolysates. In addition, certain serums and serum fractions, peptones, and peptone dialysates were used to supplement the chemically defined media in order to discover growth promoting factors. Other studies were carried out to determine if a larger amount of oxygen in the culture vessel would support a greater proliferation of freshly explanted mouse cells and the normal human diploid cell line Wi-38.

Progress: We have formulated a new medium NCTC 163 which has supported greater growth in three out of four cell lines growing in completely chemically defined medium NCTC 135. To date, however, freshly explanted cells have not been grown in medium 163 for any great period of time. The only peptone which we found satisfactory for use as a growth supplement was Witte peptone. Other peptones and peptone dialysates appeared to provide no growth promoting activity. The use of 1% oxygen in the atmosphere of cultured cells apparently helped the growth of primary mouse cells grown in Dulbecco-Vogt medium. Cells in medium NCTC 163 in 1% oxygen grew less well than in an atmosphere of 10% CO₂ in air. Wi-38 cells were not stimulated by the 1% oxygen atmosphere.

Significance for Cancer Research (NCP Objective 3 Approach 2)
and (NCP Objective 2 Approach 4.3)

In order to study malignant transformation of cells in vitro, it is necessary to be able to grow large masses of cells in as defined a growth medium as possible. In a completely defined medium, we can control the environment of the cells without running the risk of having the cells contaminated with viruses, phages, or mycoplasma which may influence the malignant transformation that occurs when cells are cultured in vitro.

Level of Effort: Professional Man-Years: 1-1/4

Serial No. NCI-3212, Project 2 SAME

1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project 2, Title: Cell transformations in vitro.

Principal Investigators: V. J. Evans and F. M. Price

Other Investigators: R.R.Gantt, R.F.Camaller, K.K.Sanford, S.L.Handleman,
and R.A.Dworkin

Cooperating Units: A. Richter, Laboratory Cell Suppliers, Frederick, Md.

Man Years: See Serial No. NCI-3211

Project Description: A study to discover differences between normal and cancerous cells and to determine factors influencing the spontaneous malignant transformation of normal cells to the neoplastic state. National Cancer Plan primarily 3.1 and secondarily 3.2.

Objectives:1) To study the phenomenon of "spontaneous" malignant transformation of cells in vitro. This type of transformation simulates human cancer.

2) To attempt to determine a factor(s) responsible for this "spontaneous" transformation.

3) To stabilize the tissue culture system so that this "spontaneous" malignant transformation does not occur, or to delay the time of onset in cells in vitro so that dynamic changes in the non-malignant or pre-cancerous cells to cancer cells may be uncovered; to make systems available so that methods to arrest the event can be explored.

Methods Employed: Experiments were planned to examine the influence of serums of different animal species (horse and bovine) and sex, and of peptones on the spontaneous neoplastic transformation of C3H_f cells in vitro. Cloned populations were obtained to explore the question of whether induction or cell selection is the primary factor in so-called malignant conversion of cells in vitro. Assessment of neoplastic conversion was made by implantation of cells into syngeneic animals and correlations were attempted by evaluation of tumor data and cytologic standards established from nonneoplastic and neoplastic cells.

Major Findings:

1. Comparative effects of mare, stallion, gelding horse serum and fetal bovine serum on neoplastic transformation in vitro.

The objective of this study was to compare the effects of mare, stallion, gelding horse, and fetal bovine serum on the spontaneous neoplastic transformation in vitro of 3 independent pools of C3H_f mouse embryo cells. Presumably, these serums would, at least, differ in hormonal content. From earlier studies, it had been determined that such cells in gelding horse serum undergo neoplastic transformation after a relatively short and predictable period in vitro, while in fetal bovine serum the transformation is both markedly delayed and less predictable in time of transformation. In this particular study the gelding horse serum was from a different source from that of the earlier studies. Further, attempts were made to diagnose the neoplastic potential of the cells by means of cytologic criteria that have been described and documented for similar cell-culture systems (cf Serial No. NCI-3213). It was found that the 3 pools varied in their potential for neoplastic transformation, with the reason still remaining unclear, although the possibilities exist that unidentified variations in explantation of the original cells or culture conditions including temperature variations or fluctuations in pH might have been influential. Another possibility is that some of the cells may have become antigenic to the syngeneic host since some cells appeared by cytologic appearance to be malignant but produced no tumors. It also appeared that mare serum, like fetal bovine serum, had a delaying or inhibiting effect on spontaneous neoplastic transformation as compared with 2 male horse serums. Cells in mare serum showed a prolonged latent period for tumor production. Also, the effect of serum on neoplastic transformation appeared to be unrelated to its growth stimulating capacity since stallion serum and fetal bovine serum were both highly growth stimulating. The results may indicate a sex hormone influence on the spontaneous transformation or that mare and fetal bovine serums may lack a potentiating factor present in the male horse serums. A manuscript has been published on this study.

2. Effect of large and small molecule fractions of mare and stallion serum on neoplastic transformation of C3H_f mouse cells in vitro.

We previously found that C3H mouse cells cultured in medium containing a small molecule fraction of gelding horse serum would be neoplastically transformed in about the same time as cells grown in whole gelding horse serum. We also found that cells grown in medium containing the large molecule of fetal bovine serum were delayed from becoming neoplastically transformed. Later, as described above, we showed that cells grown in medium containing mare serum were also delayed from producing tumors while cells grown in stallion serum were transformed relatively quickly and predictably. In this study 3 pools of C3H_f mouse embryo cells were cultured in medium containing: 1) large molecule fraction of fetal bovine serum (Lg FBS); 2) large molecule fraction of stallion serum (Lg St); 3) large molecule fraction of mare serum (Lg M); 4) Lg M plus small molecule fraction of mare serum (Sm M); 5) Lg M plus small molecule fraction of stallion serum (Sm St); 6) Lg St plus Sm St; 7) Lg St plus Sm M; 8) Lg FBS plus Sm M; and 9) Lg FBS plus Sm St.

To date transformation as evidenced by production of tumors in syngeneic hosts, has only occurred in two cell lines, both from the same embryo pool. One cell line that became transformed was that in medium containing only Lg M. The other transformed line was that in medium with Lg FBS and Sm. Both of these results were unexpected and until all tumor data are completed we are

unable to assess the significance of this transformation information. It does appear that the correlation of cytologic assay and tumor production is good. So far both transformed lines appeared transformed by our cytologic criteria. Several more cell lines now appear to be transformed cytologically, although tumors have not as yet appeared in injected hosts.

3. Influence of peptones on neoplastic transformation.

The need to substitute for serums, growth promoting factors which do not introduce agents that pass through a micropore filter is great. Various autoclavable peptones have been employed as serum substitutes for the growth of cells. Their possible influence on malignant conversion of mouse cells is under test. One C3Hf mouse embryo cell line grown in medium NCTC 135 plus 2 mg/ml Witte peptone produced tumors in syngeneic hosts after about 1 year in vitro. Two other cell lines in medium NCTC 163 supplemented with Witte peptone has been in culture for almost a year. As yet no tumors have been produced by these cells; however, recent cytologic assays have determined that these cells are neoplastic. Other cell lines in other peptones or peptone dialysates have not grown well and have not been assayed for neoplastic transformation.

4. Efforts to distinguish between induction and cell selection as they influence malignant conversion of cells in vitro.

Studies are in progress on a clone of cells originally prepared by us from pools of minced embryos of C3H cells grown in fetal bovine serum and considered to be nonneoplastic. The clones are maintained in either fetal bovine serum or stallion horse serum and are being tested for neoplastic transformation. The details of data to date are found in Serial No. NCI-3213. This entire subproject is a collaboration activity within this Section.

Significance and Proposed Course:

The data on effects of mare, stallion, and gelding horse serum and of large and small molecule fractions of gelding horse serum and fetal bovine serum upon the malignant conversion of cells raises several questions. The experiments now in progress involving the use of large and small molecule fractions of both mare and stallion serum may show if it is possible to maintain cells indefinitely without neoplastic conversion or can prolong the in vitro period in which the cells are not transformed. They may also give an indication as to the effect of hormonal influence on neoplastic transformation. Also, with appropriate fractions we will determine if there is a suppressive effect on the malignant transformation by the large molecule fraction of mare serum or fetal bovine serum. The inability to stabilize cells as non-malignant for indefinite periods hinders the effective use of cells in vitro for multiple ancillary studies, so it is highly important to resolve the factors responsible for transformation.

For some time, the quality of serums has been suspect. Serum is often collected under adverse conditions and may be contaminated with viruses and phages. Occasionally, serums contain viruses or mycoplasma which are not easily detected or removed. Also, serums may vary in protein, hormone, trace

metals, etc., from species to species or from lot to lot within the same species. It therefore has become necessary to find a replacement for whole serum which will promote growth of cells as well as serum does but is more defined in chemical nature and can be easily obtained. Ideally, a completely chemically defined growth-promoting factor should be used. To this end, we are investigating the use of peptones and/or substances derived from them. We shall continue studies in progress to determine whether peptones can be used as a medium supplement in place of serums. Further studies will encompass isolation and purification of definite substances from peptones which supplement the media (see also Serial No. NCT-3257). In this way it will be possible to produce uniform media to be used in studies involving "spontaneous" neoplastic transformation of mammalian cells.

To resolve the question of whether the serum effect is an induction phenomenon or the result of cell selection, establishment of clonal derivatives from non-malignant cells is required. From a recently isolated non-malignant clone, some sublines have been continued in fetal bovine serum and in gelding and stallion horse serum. The data from this experiment should tell us whether the cells in fetal bovine serum have selected cells of a type unlikely to undergo malignant transformation or whether fetal bovine serum stabilizes cells in a state more nearly approaching normality. If cells from this clone when subjected to horse serum become transformed, then we can infer that the horse serum operates to induce transformation, since selection is ruled out by use of derivatives of a single cell. If the cells do not transform, then we must assume that earlier mixed cell populations which became neoplastic were ones which were selected by the horse serum which were most likely to be transformed.

The obvious usefulness of characterized non-malignant and malignant cell lines from a single cell in many projects is far reaching, particularly for comparative biochemical studies.

Honors, Awards, and Other Activities (Dr. V. J. Evans):

1. Vice-President, Tissue Culture Association, 1968-1972.
2. President, Tissue Culture Association, 1972-1974.
3. Recipient of Superior Service Award, Department of Health, Education and Welfare, 1972.
4. Member, Scientific Advisory Board, Institute for Medical Research, Camden, N. J., 1973.
5. Consultant for Index of Tissue Culture, prepared by National Library of Medicine; literature in field from 1966-1970 published by October House; 1971-1972 published by Tissue Culture Association and printed by Waverly Press.
6. Member, ex officio, all Standing Committees excluding Nominations Committee, of Tissue Culture Association, 1972-1974.

7. Invitation to prepare section on Replicate Culture Methods. I. Monolayer Cultures, in Methods and Applications of Tissue Culture, eds., P. F. Kruse and M. K. Patterson, Academic Press, Inc., New York, 1972.
8. Invitation to Dr. W. G. Taylor and Dr. Evans to prepare a chapter on Preparation and Use of Mammalian Cell Cultures, in Methods in Cell Physiology, Vol. 8, ed. D. M. Prescott, Academic Press, Inc., New York, 1973.
9. On-site evaluation for 3-week course on Cell Culture for Aging Research at Department of Microbiology, University of Vermont Medical School, project of Adult Development and Aging Branch, National Institute of Child Health and Human Development, NIH, 1972.
10. Co-organizer of Workshop on Contaminations in Cell Cultures and Problems of Serum Quality Control., W. Alton Jones Cell Science Center, April 24-26, 1973.
11. Consultant to Interagency Board, U. S. Civil Service Commission.

Publications:

1. Taylor, W.G., Price, F.M., Dworkin, R.A., and Evans, V.J.: A simple device for planting replicate mammalian cell cultures. In Vitro 7: 295-299, 1972.
2. Fedoroff, S., Evans, V.J., Hopps, H. E., Sanford, K.K., and Boone, C.W.: Summary of Proceedings of a Workshop on Serum for Tissue Culture Purposes. In Vitro 7: 161-167, 1972.
3. Evans, V.J., and Taylor, W.G.: Replicate Culture Methods: Monolayer Cultures in Methods and Applications of Tissue Culture, eds., P.F.Kruse and M.K.Patterson, Academic Press, Inc., New York, 1972.
4. Evans, V.J., Price, F.M., Sanford, K.K., Kerr, H.A., and Handleman, S.L.: Comparative effects of mare, stallion, gelding horse, and fetal bovine sera on neoplastic transformation in vitro. J Nat Cancer Inst 49: 505-512, 1972.
5. Taylor, W.G., Dworkin, R.A., Pumper, R.W., and Evans, V.J.: Biological efficacy of several commercially available peptones for mammalian cells in culture. Exp Cell Res 74: 275-279, 1972.
6. Richter, A., Sanford, K.K., and Evans, V.J.: Influence of oxygen and culture media on plating efficiency of some mammalian tissue cells. J Nat Cancer Inst 49: 1705-1712, 1972.
7. Gantt, R.R., Montes de Oca, F., and Evans, V.J.: Methylation of satellite DNA in mouse neoplastic and nonneoplastic cell cultures. In Vitro 8: 288-294, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3212, Project 2

Title: Cell Transformations in vitro.

Principal Investigator: V.J.Evans,K.K.Sanford,R.R.Gantt,W.G.Taylor,F.M.Price

Section: Tissue Culture Section

Lab/Branch: Laboratory of Biology

Goal: To discover differences between normal and cancerous cells and to determine factors influencing the spontaneous malignant transformation of normal cells to the neoplastic state.

Approach

Experiments were planned to examine the influence of mare, stallion, and gelding horse serum, and large and small molecule fractions of mare and stallion horse serum, and of peptones on the spontaneous neoplastic transformation of C3H mouse cells in vitro. Assessment of neoplastic conversion is being made by implantation of cells into syngeneic hosts. We also are attempting to correlate tumor data with cytologic data established from previous studies with nonneoplastic and neoplastic cells. Cloned populations of cells have been obtained to explore the question of whether induction or cell selection is the primary factor in malignant transformation of cells in vitro.

Progress:

We have determined that cells in stallion horse serum transform in a relatively short and predictable period in vitro, as do cells in gelding horse serum. Mare serum and fetal bovine serum appear to delay the onset of neoplastic transformation. We have not yet determined the effect of large and small molecule fractions of mare and stallion serum on neoplastic transformation, although 3 separate confirming experiments are in progress. Cells grown in medium containing Witte peptone as a supplement have undergone transformation after approximately one year in culture.

Significance for Cancer Research (NCP Objective 3 Approach 1)

These studies are made in an effort to find a way to prevent mammalian cells in vitro from becoming neoplastic spontaneously or to determine factors responsible for that transformation. If we can achieve the former, then we can treat the cells chemically, physically, with viruses, etc., and possibly determine factors responsible for malignancy and also possibly control the transformation. Clearly, data concerning malignant transformations of cells in vitro must be evaluated in concert with the known fact that as yet uncontrolled "spontaneous" Level of Effort: Professional Man-Years: transformations take place.

1-1/4

Serial NO. NCI-3213, Project 1 SAME

1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project 1, Title: Carcinogenesis in vitro.

Principal Investigator: K. K. Sanford

Other Investigators: S.L.Handleman, M.L.Hursey, G.M.Jones, C.U.Garrison,
R.W.Tucker, T.P.Yang, V.J.Evans, R.R.Gantt, W.G.Taylor

Cooperating Units: R. Parshad, Department of Pathology, Howard University
School of Medicine, Washington, D.C.;
A. Richter, Laboratory Cell Suppliers, Frederick, Md.;
J.W. Hartley, Laboratory of Viral Diseases, NIAID, NIH.

Project Description:

Objectives:

1. To elucidate the cause(s) of "spontaneous" neoplastic transformation of mammalian tissue cells in vitro.
2. To understand the serum influence on spontaneous neoplastic transformation.
3. To examine the effects of a chemical carcinogen on skin cells from normal humans and those with genetic defects and high cancer risk.
4. To improve plating efficiency and clonal growth of mammalian tissue cells for carcinogenesis studies.
5. To improve the quantitative assay for carcinogenesis by chemical carcinogens.
6. To explore the effects of chemical carcinogens on neoplastic transformation and cytology of cells in culture.

Methods Employed: General methods employed will be described under each study.

Study 1.--Possible causes of "spontaneous" neoplastic transformation of mammalian tissue cells in vitro.

A. Endogenous viruses of murine leukemia-sarcoma complex. This study, detailed in last year's annual report, was published (see Publications).

B. Chromosomal aberrations. A study of the relationship between chromosomal aberrations, type of serum, and neoplastic transformation in cells from 3 inbred strains of mice was published (see Publications).

C. DNA damage and impaired DNA repair. (See Project NCI-3264 project 3).

Study 2.--The serum influence on neoplastic transformation.

A. Mechanism of the transformation. Does the effect of stallion or gelding horse serum in accelerating spontaneous neoplastic transformation result from selection of cell types more susceptible to transformation, or does some factor(s) in the serum induce transformation? In attempts to answer this question, we are currently exploring the effects of horse serum and fetal bovine serum on 5 clones derived from single cells of nonneoplastic mouse cell lines.

B. Chemical analyses of sera influencing neoplastic transformation. This study, detailed in last year's annual report, was published (see Publications).

C. Evidence for an inhibiting influence of fetal bovine serum on "spontaneous" neoplastic transformation in vitro. This study, detailed earlier, was published (see Publications).

D. Effect of serum fractions on neoplastic transformation (see Serial No. NCI-3212, project 2).

Study 3.--Effects of benzo(a)pyrene (BP) on normal and mutant human cells in vitro. (Collaboration with R. Parshad).

This study was undertaken with the following objectives: 1) to ascertain whether skin fibroblasts from individuals with genetic disorders associated with high cancer risk could be morphologically transformed by BP more readily than control cells from normal individuals; 2) to ascertain whether any of these cells underwent chromosomal alterations and could be established as continuous cell lines. Skin fibroblasts from patients with Fanconi's anemia, ataxia telangiectasia, xeroderma pigmentosum, basal cell nevus syndrome, and Down's syndrome and from normal individuals including a sib of the xeroderma patient and CRL 1121 from the ATCC were grown in Dulbecco-Vogt's medium + 10% fetal bovine serum. Cells were treated for six weeks with 20 ug/ml BP or with the solvent 0.2% acetone in the medium. All cell lines, except those from the xeroderma patient, showed a decline of growth after 24-41 transplant generations (217-365 days) in vitro. The lines from the xeroderma patient declined in growth earlier after 15-16 transplant generations (147 days) in vitro. Ultimately all cell lines died exhibiting a finite life in vitro. There was no definitive cytologic manifestation of neoplastic transformation in any of the cell lines although a number of lines irrespective of source or treatment showed some variation in cell size and loss of parallel orientation. Most of the cell lines became heteroploid long before the decline in growth. These results indicate that under present culture conditions, human heteroploid cells may exhibit a finite life in culture as previously shown for human diploid cells and that a shift from diploidy is not a sufficient condition for establishment of a continuous human cell line.

Study 4.--Factors influencing plating efficiency and single cell growth in vitro. (Collaboration with A. Richter).

Clones from single cells taken directly from normal tissues or from cultures of nonneoplastic cells, as well as more effective plating techniques

are needed for studies on carcinogenesis in vitro. In an effort to develop more nearly optimal conditions for the growth of single nonneoplastic tissue cells, we have examined the influence of certain physico-chemical factors on the plating efficiency of cells from freshly isolated tissues and from short and long-term culture. The first study detailed in last year's report was published (see Publications). These studies demonstrated improved colony formation of a variety of mammalian cell lines as well as primary cells when a gas atmosphere of low oxygen, carbon dioxide, and nitrogen was used, compared to the usual atmosphere of carbon dioxide in air. It was shown that the toxic component of the CO₂:air mixture was oxygen and that the optimal concentration of oxygen in the gas phase for growth and plating of single cells was between 1-3%. The present studies extend these observations to African green monkey kidney strain Vero and human diploid lung strain WI-38. A relation between pH and oxygen tension was found to influence the growth rate and plating efficiency of the cells. Cells were more susceptible to oxygen toxicity at alkaline pH than at pH 7.0. Some cell lines were susceptible to oxygen toxicity at neutral and alkaline pH; other cell lines were susceptible only at alkaline pH. The latter more resistant lines may have acquired partial oxygen resistance during long-term culture. It is possible that the relation between oxygen and pH shown to influence clonal growth may apply to growth of mass cultures.

Study 5.--The quantitative assay for "transformation" by chemical carcinogens.

Repeated efforts in this laboratory (as well as some other laboratories) to obtain reproducible quantitative morphologic responses to chemical carcinogens have not been successful. We are currently analyzing several factors that we think may influence this assay. We wish to adapt this assay to mouse and human cells to explore: 1) the possibility of using this system for detecting effects of serum fractions on mouse cells, and 2) the comparative response of normal human cells and those from individuals having genetic defects associated with high cancer risk.

Study 6.--Effects of chemical carcinogens on neoplastic transformation and cytology of cells in culture.

In preliminary studies, single or repeated short (1- 2 $\frac{1}{2}$ hour) exposures of cells to 10⁻⁶ M 4-nitroquinoline-1-oxide (NQO) or continuous exposure to 1 mM N-methyl-N-nitrosourea for 19 days failed to induce neoplastic transformation or enhance spontaneous transformation in recently explanted cells or long-term lines from the mouse, Syrian hamster, Chinese hamster, or rat. Spontaneous neoplastic transformations occurred during the experiments in mouse and Chinese hamster cells. In 4 series, secondary or tertiary cultures of Syrian hamster embryo cells were exposed for longer periods to 10⁻⁶M NQO or 1 ug/ml 3-methylcholanthrene. Growth studies following treatment suggested that the carcinogens promoted the establishment of continuous cell lines. More carcinogen-treated cell lines underwent neoplastic transformation than did control cells and this occurred earlier in the treated lines. However, the neoplastic response to carcinogen was neither rapid nor completely reproducible. Cytologic manifestations of neoplastic transformation were described and documented. The transformed cells were usually neither fusiform nor oriented in crisscross growth pattern, a morphology used as the marker of

transformation in certain quantitative assays. These observations raise questions concerning the stability of this morphologic marker, its applicability to diverse cell types, and its relationship to neoplastic transformation. This study is being prepared for publication.

Proposed Course:

Study 1C will be continued in an effort to correlate DNA damage and impaired DNA repair with neoplastic transformation produced by horse serum fraction. If successful, effects of fetal bovine serum fractions in inhibiting phenomenon will be examined.

Studies 2A,D will be continued to attain objectives listed. An extension of study 2B is a series of experiments on the effect of zinc on spontaneous neoplastic transformation. This study will be completed.

Studies 4 and 5 will be continued to attain the objectives listed.

Honors, Awards, and Other Activities:

1. Trustee of the Tissue Culture Association, Inc., and member of the Council
2. Member, Advisory Committee of the Animal Cell Culture Collection of the American Type Culture Collection
3. Member, Editorial Advisory Board of Cancer Research
4. Member, Terminology Committee, Tissue Culture Association
5. Invitation to lecture at Indiana University School of Medicine

Publications:

1. Sanford,K.K, Jackson,J.L., Parshad,R., and Gantt,R.R.: Evidence for an inhibiting influence of fetal bovine serum on spontaneous neoplastic transformation in vitro. J Nat Cancer Inst 49: 513-518, 1972.
2. Sanford,K.K., Handleman,S.L., Hartley,J.W., Jackson, J.L., and Gantt,R.R.: "Spontaneous" neoplastic transformation in vitro: Influence of endogenous murine leukemia virus and serum fractions. J Nat Cancer Inst 49: 1177-1189, 1972.
3. Sanford,K.K., Burris,J.F., and Handleman,S.R.: Chemical analyses of sera influencing neoplastic transformation in vitro. J Nat Cancer Inst 49: 1553-1561, 1972.
4. Evans,V.J., Price,F.M., Sanford,K.K., Kerr,H.A., and Handleman,S.L.: Comparative effects of mare, stallion, gelding horse, and fetal bovine sera on neoplastic transformation in vitro. J Nat Cancer Inst 49: 505-512, 1972.
5. Parshad,R. and Sanford,KK: Search for genetic influences on neoplastic transformation and chromosome stability of mouse cells cultured in horse or fetal bovine serum supplemented medium. J Nat Cancer Inst 49: 1155-1163, 1972.

6. Richter,A., Sanford,K.K., and Evans,V.J.: Influence of oxygen and culture media on plating efficiency of some mammalian tissue cells. J Nat Cancer Inst 49: 1705-1712, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3213, project 1.

Title:

Carcinogenesis in vitro.

Principal Investigator: K.K.Sanford, S.L.Handleman, M.L.Hursey

Section: Tissue Culture Section

Lab/Branch: Laboratory of Biology

Goal: To elucidate cause(s) of neoplastic transformation of cells in vitro and to improve quantitative assays for in vitro carcinogenesis.

Approach Experiments have been carried out or are in progress to test certain hypotheses concerning the malignant transformation that occurs quite regularly during culture of mammalian tissue cells in the absence of deliberately added carcinogens or viruses. These hypotheses include expression of endogenous viruses of the leukemia-sarcoma complex, chromosomal aberrations and breaks, and carcinogenic and anti-carcinogenic effects of certain serum fractions. Conditions that facilitate rapid, controlled, and reproducible neoplastic transformation in clones of stable nonneoplastic cells are being analyzed in experiments with chemical carcinogens and experiments testing physico-chemical influences on single cell growth.

Progress: The lack of correlation between presence of gs antigens of endogenous murine leukemia virus (MuLV) and neoplastic transformation in 48 mouse cell lines suggest that these transformations may not be caused by MuLV. The diverse effects of male horse serum and fetal bovine serum on both neoplastic transformation and chromosomal change were extended to cells from 2 or 3 other inbred mice. The low molecular weight fraction of horse serum has been implicated in accelerating and fetal bovine serum components in inhibiting neoplastic transformation.

Growth of single cells for plating assays was increased by reducing oxygen levels. The morphologic marker used as evidence of "transformation" in quantitative assays did not characterize the neoplastic cell lines in our studies indicating that further cytologic study is needed for this assay. Significance for Cancer Research (NCP Objective 3 Approach 2)

These malignant transformations in vitro are probably analogous to those occurring spontaneously in man. Elucidation of causes and influencing factors should help in understanding the cellular events in oncogenesis. The quantitative in vitro assay may have applicability in environmental screening for carcinogens.

Level of Effort: Professional Man-Years: 2/3

1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project 2, Title: Manifestations of neoplastic transformation in vitro.

Principal Investigator: K. K. Sanford

Other Investigators: S.L.Handleman, G.M.Jones, M.L.Hursey, C.U.Garrison,
R.W.Tucker, R.L.Steinberg.

Cooperating Units: C.C.Ting, DCBD, NCI
B.K.Wetzel, Dermatology, NCI

Project Description:

Objective: To identify any characteristics of cells in culture that may be directly or indirectly associated with their neoplastic transformation in vitro.

Methods Employed: General methods will be described under each study.

Study 1.--A comparative cytologic study of mouse, rat, hamster, and human fetal cells during neoplastic transformation and/or long-term culture in vitro.

The objective is to establish more quantitative, objective cytologic criteria for neoplastic transformation of cells in vitro and a systematic approach to diagnoses of neoplastic state.

This study,detailed in last year's annual report, is being completed as in vivo assay results are becoming available.

Study 2.--Fetal antigens in cells undergoing neoplastic transformation in vitro. Collaboration with Dr. C. Ting.

The objective is to study the expression of fetal antigens in cultures of mouse cells during early culture, late culture before neoplastic transformation, and late culture after neoplastic transformation.

The details of this project were presented in last year's annual report. Further results indicate that C3H mouse fetal cells gradually lost fetal antigens during long-term culture and no new ones have been detected as a result of neoplastic transformation. C57BL fetal cells retain some expression of fetal antigens after neoplastic transformation and these are being compared with those of early passage.

Study 3.--Cinematographic and scanning electron microscopy study of a pair of neoplastic and nonneoplastic cell lines of common single cell origin. Collaboration with B. Wetzel.

The objective is to compare the dynamic morphology and surface membrane changes in nonneoplastic and neoplastic cells derived from the same cell.

This project has just recently been initiated.

Study 4.--Immunologic discrimination between neoplastic and nonneoplastic cells in vitro. See Serial No. NCI-3264, project 2.

Proposed Course: These 4 studies will be continued to attain the objectives listed.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3213, project 2.

Title:

Manifestations of neoplastic transformation in vitro.

Principal Investigator: K.K.Sanford, S.L.Handleman, M.L.Hursey, G.M.Jones.

Section: Tissue Culture Section

Lab/Branch: Laboratory of Biology

Goal: To identify any characteristics of cells in culture that may be directly or indirectly associated with their neoplastic transformation in vitro.

Approach The studies include: 1) A comparison of cytologic changes in cultures of fetal cells of human, rat, mouse, and hamster origin during neoplastic transformation and/or long-term culture, in order to establish cytologic criteria for in vitro diagnosis of neoplastic state; 2) The expression of fetal antigens in cultured cells during early passage, late passage before neoplastic transformation, and late passage after neoplastic transformation to detect any specific antigenic changes associated with neoplastic transformation; 3) A comparison of the dynamic morphology and surface membrane changes in neoplastic and nonneoplastic cells originating from the same cell; 4) Immunologic discrimination between neoplastic and nonneoplastic cells in culture.

Progress: 1) Cytologic criteria of neoplastic transformation previously described and validated for C3H mouse cells in culture appear with few exceptions to be applicable and valid for fetal cells from the hamster, rat, and human. Some additional criteria have been found useful and the relative importance of the diverse criteria have been reevaluated in the development of a systematic guide for diagnosis. 2) C3H mouse fetal cells were found to lose fetal antigens during long-term culture and no new ones have been detected after neoplastic transformation. A slightly different response in C57BL fetal cells is being studied. 3) A pair of neoplastic and nonneoplastic cell lines derived from the same cell have been developed and are being studied for dynamic morphology and alterations in surface membranes. 4) Assay techniques for selective cytotoxicity of peritoneal leukocytes for neoplastic cells in vitro are being developed. Significance for Cancer Research (NCP Objective 3 Approach 2) /oped. (see 326)

Any cellular changes regularly associated with neoplastic transformation are of fundamental importance in understanding cancer.

Level of Effort: Professional Man-Years: 3.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Primary biochemical alterations leading to neoplastic conversion.

Principal Investigator: R.R.Gantt

Other Investigators: V.J.Evans, B.T.Julian, G.H.Smith

Project Description: A program to elucidate the primary chemical alterations which are necessary biochemical changes leading to neoplastic transformation and to devise methods to detect and control the transformation based on these chemical alterations.

Objective: To identify primary changes in the methylation of cellular and viral nucleic acids during neoplastic transformation and to exploit these changes to develop an assay for, and possible control of, neoplastic transformation in vitro.

Methods Employed:

1. In order to identify primary changes in the methylation of nucleic acids, significant differences are first being established before and after neoplastic conversion. These differences will then be correlated with conversion time and with the method of conversion (i.e., viruses, chemical carcinogens, or the horse serum-fetal bovine serum system). These differences are monitored by double isotope co-elution chromatography and enzyme analysis.

2. The discovery of N^2 -guanine RNA methyltransferase in AMV has suggested that other oncogenic RNA viruses may have the same or similar activities associated with the virion. Therefore, available viruses have been examined for RNA methylase activities.

Major Findings:

1. The C-type sarcoma viruses have two methylase activities (N^2 -methylguanine and 1-methyladenine), while the leukemogenic viruses have only one activity. (Gantt, Smith, Julian, Virology 1973).

2. Demonstrated that the N^2 -methylguanine methylase of AMV is not associated with the viral core after disruption of the virion by specified reagents. (Gantt, Stromberg, Julian, J. Virology 1972).

3. Purified and characterized the N^2 -guanine RNA methyltransferase associated with the AMV. (M.Taylor, Gantt, manuscript in preparation).

4. In contrast to the C-type RNA tumor viruses (AMV, RSV, RAV-1, MSV, MLV) the MTV virion contains no detectable RNA methylase activity, but the intracytoplasmic A particles, invariably associated with B particles budding from the membrane, have N²-guanine methylase activity.
5. Developed a rapid low pressure chromatographic assay for RNA methylase (Gantt, Julian, Anal. Bioch., 1973), and recently a high pressure, high resolution chromatographic assay (Gantt, Julian, in preparation).
6. Demonstrated that 5-methylcytosine is equally distributed on both strands of mouse satellite DNA. (Gantt, Montes de Oca, Evans, In Vitro, 1973).
7. Discovery of DNA methylase activity associated with the DNA replicating complex of mammalian cells. (Longfellow, Gantt, Smith).

Significance:

The discovery of a specific RNA methylase associated with C-type oncogenic viruses adds further support to the idea that aberrant methylation may be a fundamental event leading to neoplastic transformation. The fact that the mouse mammary tumor virion is lacking the methylase activity is offset by the enzyme's presence in the intracytoplasmic A particles which are always present when the B particles are budding from the membrane.

The finding that both strands of DNA are equally methylated and that the DNA methylase is found in the replication complex suggests that the functions proposed to date (endonuclease restriction and/or conversion to an initiator codon) are not satisfactory. We have proposed that there is more than one kind of DNA replication system and that 5-methylcytosine patterns serve as recognition sites.

Proposed Course of Investigation:

1. Establish whether the N²-guanine methylase is essential for the virus life cycle.
2. Identify the differences in the paired cell lines using the newly developed methods.
3. Determine whether the mammalian DNA requires methylation to serve as a template for replication.

1972-1973 Publications:

1. R.R.Gantt, K.J.Stromberg, B.T.Julian: Absence of ribonucleic acid methylase in the avian myeloblastosis virus core. J. Virology 9: 1057-1058, 1972.
2. K.K.Sanford, J.L.Jackson, R.Parshad, R.R.Gantt: Evidence for an inhibiting influence of fetal bovine serum on spontaneous neoplastic transformation in vitro. J. Nat. Cancer Inst. 49: 513-518, 1972.
3. K.K.Sanford, S.L.Handleman, J.W.Hartley, J.L.Jackson, R.R.Gantt: "Spontaneous" neoplastic transformation in vitro: Influence of endogenous murine leukemia virus and serum fractions. J Nat Cancer Inst 49: 1177-1189, 1972.

4. R.R.Gantt, F.Montes de Oca, V.J.Evans: Methylation of satellite DNA in mouse neoplastic and nonneoplastic cell cultures. In Vitro 8: 288-294, 1973.
5. R.R.Gantt, B.T.Julian: A rapid column chromatographic procedure for the assay of RNA methyltransferases. Anal. Biochem. accepted for publication and in press, 1973.
6. R.R.Gantt, G.H.Smith, B.T.Julian: Base specific methylase activity in RNA tumor viruses: Avian leukosis virion-associated RNA methylase(s). Virology, accepted for publication and in press, 1973.
7. K.J.Stromberg, R.R.Gantt, S.H.Wilson: Structural studies on avian myeloblastosis virus: Conditions for isolation and biochemical characteristics of the core component. Biochim. Biophys. Acta, 304: 1-11, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3234A

Title: Primary biochemical alterations leading to neoplastic conversion.

Principal Investigator: R.R.Gantt; also G.H.Smith, V.J.Evans, B.T.Julian

Section: Tissue Culture Section

Lab/Branch: Laboratory of Biology, DCB&D, NCI

Goal: To identify primary changes in the methylation of cellular and viral nucleic acids during neoplastic transformation and exploit these changes to develop an assay for, and possible control of, neoplastic transformation in

Approach In vitro.

Nucleic acid methylation is involved in cell regulation, control, and probably differentiation. Numerous studies indicate that aberrant nucleic acid methylation is involved in the fundamental events leading to neoplastic transformation. Two general kinds of experiments are employed to investigate this hypothesis in depth: (A) Controlled comparisons of cell-free extracts for the presence of enzyme activities after cell breakage and separation. (B) Controlled comparisons of the enzyme activities in the intact cell by analysis of the methylated components produced during cell growth or turnover. The biochemical techniques employ double isotope experiments to attain the requisite sensitivity and to eliminate technical and procedural artifacts.

Progress:

- 1) The C-type sarcoma viruses have two methylase activities (N^2 -guanine and 1-methyladenine) while the leukemogenic viruses have only one activity (N^2 -methylguanine).
- 2) In contrast to the C-type RNA tumor virions (AMV, RSV, RAV-1, MSV, MLV) the B-type virion (MTV) contains no detectable RNA methylase activity. However, the intracytoplasmic A particle associated with MTV appears to contain N^2 -guanine methylase.
- 3) An assay nearly 100-fold more sensitive has been developed which is quantitative and specific for the methylated bases.

Significance for Cancer Research (NCP Objective 3 Approach 2)

If aberrant methylation is a fundamental event in all conversion, it should be feasible to design a highly specific chemotherapeutic agent focused on a subtle level of cellular control (compared with cell division) and possibly provide a method for detecting the conversion rapidly.

Level of Effort: Professional Man-Years: 1.

Serial No. NCI-3251

1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Testing for possible carcinogenic action of Enovid in mice

Principal Investigator: W. E. Heston

Other Investigators: G. Vlahakis

Project Description: Objective 1. Approach 1.

Objectives: The aim of this study was to ascertain whether or not the antifertility drug, Enovid, so widely used in women, has any carcinogenic effect when fed to female mice in doses up to that which prevents them from reproducing.

Methods: The Enovid was fed continuously in the NCI mouse food in three doses:

- 5 µg Enovid/g food (all females on this dose could breed)
- 10 µg Enovid/g food (some of the females on this dose could breed)
- 20 µg Enovid/g food (all females on this dose were sterile)

The highest dose was comparable on a weight basis to the dose given women.

The Enovid was fed to females of five especially selected strains:

- C3H - for testing induction of mammary tumors
- C3HfB - for testing induction of mammary and ovarian tumors
- A - for testing induction of mammary tumors
- BALB/c - for testing induction of cervical, vaginal, and adrenal tumors
- C57BL - for testing induction of hypophyseal tumors

Significance of the Program to the Institute:

It was essential that an extensive testing of the effect of this antifertility drug especially on possible induction of tumors be made in the dose range that prevented fertility. This was because of the wide spread use of these drugs in women throughout the world. It is significant that the only real increase in tumors was in regard to hypophyseal tumors in old females. This suggests that it would be well to collect hypophyses at autopsy of women who have been on the "pill" and send them to a central pathology laboratory where they could be examined for possible early neoplastic changes.

Proposed Course of Experiment:

These vaginal changes that occur in the BALB/c females are very interesting for they suggest that BALB/c might be an appropriate model for studying further in experimental animals the occurrence of carcinomas of the vagina in young females whose mothers were treated with estrogen to maintain pregnancy. The study will now take a course in this direction.

Publication:

Heston, W. E., Vlahakis, G., and Desmukes, B.: Effects of the anti-fertility drug Enovid in five strains of mice with particular regard to carcinogenesis. J. Natl. Cancer Inst. 51: , 1973.

Results: Final tabulation and analysis of the data showed the following:

Inbred strains of mice differ in their response to Enovid.

All female mice continued to breed when given 10 $\mu\text{g/g}$ food, and all were sterile when given 20 $\mu\text{g/g}$ food. Preliminary testing showed no genetic charges in offspring of females fed Enovid. Females that became pregnant when fed Enovid tended to abort more often than controls.

Treatment with Enovid reduced the gain in weight in all strains in proportion to the doses given.

In one strain Enovid treatment had no effect on life span, in two strains life span was lengthened, and in two life span was shortened because of the effect on tumors or other lesions in these strains.

Observations on tumor induction are noted according to type of tumor for which strains are especially susceptible.

Strain C3H: There was no increase in mammary tumors in Enovid treated females.

Strain C3HfB: There was no increase in mammary tumors or in ovarian tumors in Enovid treated females. There was an inhibition of hepatomas in the females treated with Enovid.

Strain A: There was no increase in mammary tumors in Enovid treated females.

C57BL: There was a significant increase in hypophyseal tumors in old females that had been treated with Enovid at the sterilizing dose.

BALB/c: The previously reported vaginal lesions were observed in control females as well as Enovid treated females in stages through Gardner's grade 3 with those on the highest dose of Enovid showing possibly further progression than in the controls, but none were definite neoplasms of Gardner's grade 4 showing invasion beyond the wall of the vagina or metastases. There was some inhibition of adrenal cortical adenomas in Enovid treated females of this strain.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3251

Title: Testing for possible carcinogenic action of Enovid in mice

Principal Investigator: W. E. Heston

Section: General Biology Section

Lab/Branch: Laboratory of Biology

Goal: To determine whether the antifertility "pill" Enovid is carcinogenic when administered to mice in the sterilizing dose.

Approach: The drug was administered continuously in the food in three doses:

5 $\mu\text{g/g}$ food (with which all females still could breed)

10 $\mu\text{g/g}$ food (with which some of the females still could breed)

20 $\mu\text{g/g}$ food (with which all the females were sterile)

It was administered to females of five different strains chosen because of their susceptibility to specific tumors.

C3H - for testing for induction of mammary tumors

C3HfB - for testing for induction of mammary and ovarian tumors

A - for testing for induction of mammary tumors

C57BL - for testing for induction of hypophyseal tumors

BALB/c - for testing for induction of cervical and vaginal tumors and adrenal cortical adenomas

Progress: The work has been completed, written up and accepted for publication.

The only tumors definitely increased with Enovid treatment were the hypophyseal tumors. The vaginal lesions may have shown some further progression with the treatment. There was some inhibition of hepatomas and adrenal cortical adenomas with the Enovid treatment.

Significance for Cancer Research (NCP Objective 1 Approach 1)

It was essential to have a more thorough testing for carcinogenicity in experimental animals of this drug that is so widely administered to women. Additional significance of the work was that it revealed BALB/c as a possible model for further study in experimental animals of induction of carcinoma of the vagina in young females whose mothers were treated with estrogen to maintain pregnancy.

Level of Effort: Professional Man-Years: (Total) 2

Serial No. NCI-3254

1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: An Ultrastructural and Biochemical Characterization of Avian Myeloblastosis Virus (AMV). I. Isolation of the Core Component. II. Identification and Localization of Enzymatic Capacities in the Virion. III. RNA and Polypeptide Composition of the Core Component. IV. Ultrastructural Characterization of Virion and Core

Principal Investigator: Kurt J. Stromberg

Man Years:

Total:	4
Professional:	1
Other:	3

Project Description:

The application of a new surfactant for isolation of the core of avian myeloblastosis virus and the development of optimal methods for its use has opened the way to characterization of the outer envelope and inner core structure which are the two major subcomponents of AMV, a representative type-C particle of the RNA tumor viruses. By surfactant-mediated removal of the envelope constituent of AMV, an assessment of the core constituent in ultrastructural and biochemical terms is under way.

National Cancer Plan: Objective 5, Approach 4

Methods and Results:

The experimental design has been to fractionate AMV into its component parts by two successive methods: firstly, by exposure of intact AMV to a nonionic surfactant which separates the viral "envelope" from the viral "core"; and secondly, by exposure of the resulting viral cores to high concentrations of sodium chloride which releases the inner viral "nucleoid." The three viral components are separated by ultracentrifugation and then characterized by electron microscopic, biochemical, and enzymatic methods.

Nonionic surfactant treatment with Sterox SL of AMV purified by rate-zonal centrifugation in Ficoll gradients permits isolation of a core component with a buoyant density of 1.27 g/cc in sucrose gradients. Use of a phosphate buffer instead of a Tris-HCl buffer during treatment enhances the yield of core protein. Core yield is linear over a range of starting intact AMV between 0.75 mgm/ml and 2.25 mgm/ml when the final concentration of Sterox SL is 1.5% (v/v). A five-fold increase in specific activity of endogenous RNA-dependent DNA polymerase is found in core preparations, while no activity of a recently described specific RNA methylase activity is detected. The core component of AMV contains 86% protein, 8% RNA, and 6% phospholipid as compared to 62% protein, 3% RNA and 35% phospholipid in the virion of AMV. The AMV core does not contain measurable glycoprotein or a major 22,000 M.W. structural polypeptide which are found in intact AMV preparations. The presence of most of the transfer RNA (tRNA) in the core component, in addition to the 60-70S RNA genome, has been determined, and suggests that tRNA species may be involved in the viral life cycle. SDS-polyacrylamide gel electrophoresis indicates the AMV core does not contain glycoprotein, or a major 22,000 M.W. polypeptide which are found in intact preparations of AMV. Polypeptides of 91,000 and 64,000 M.W. appear to correspond to the two subunits detected in preparations of purified AMV DNA polymerase; this, then, identifies which of the approximately 20 proteins present in intact AMV are responsible for reverse transcriptase activity. Our studies indicate that a major 14,000 M.W. polypeptide is the core protein in association with the 60-70S RNA genome of the virus.

Recent work has focused on a method for selective release and partial purification of the DNA polymerase activity of the core component of AMV by exposure to sodium chloride. A greater than 10-fold purification is rapidly achieved in this manner which releases in addition those several polypeptides which appear to comprise the inner "nucleoid" of the AMV core.

Significance of this Project to the Institute:

Rapid, simple, and quantitative methods have been developed for separation of the envelope "core" and nucleoid components of a representative C-type RNA tumor virus. Characterization by ultrastructure, biochemical, and enzymatic means of animal type-C viruses is a prerequisite to a search for similar viral-like information in human fluids and precancerous tissues. As an example, the unique and important advantage of salt treatment of AMV cores is that the DNA polymerase-containing core is separated from possible non-viral contaminants and membrane components before release of the DNA polymerase by sodium chloride. This procedure, therefore, may assist in the detection of viral particle-associated DNA polymerase activity in crude biological materials such as tumor extracts, samples of milk, etc.

In summary, these procedures of fractionation and characterization can now be applied on human material to glean information on the association of type-C viruses to cancer in man. Because the enzymatic and polypeptide assays can be made to be highly specific and extremely sensitive, it may be possible to develop tests for early detection of cancer in high-risk human populations.

Publications:

Stromberg, K., Litwack, M., and Desmukes, B.: Structural studies on avian myeloblastosis virus: Rapid purification and quantitation. Proc. Soc. Exptl. Biol. Med. 141: 215-221, 1972.

Gantt, R. R., Stromberg, K., and Julian, B.: Absence of ribonucleic acid methylase in the avian myeloblastosis virus core. J. Virol. 9: 1057-1058, 1972.

Stromberg, K., Gantt, R., and Wilson, S. H.: Structural studies on avian myeloblastosis virus: conditions for isolation and biochemical characteristics of the core component. Biochim. Biophys. Acta 304: 1-11, 1973.

Stromberg, K. and Wilson, S. H.: Release of DNA polymerase activity and selective polypeptides from the core component of avian myeloblastosis virus by exposure to sodium chloride. Proc. Amer. Assoc. Cancer Res. 14: 122, 1973.

Stromberg, K. and Litwack, M.D.: Structural studies of avian myeloblastosis virus: presence of transfer RNA in core component. Biochim. Biophys. Acta, in press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3254

Title: An Ultrastructural and Biochemical Characterization of Avian Myeloblastosis Virus (AMV)

Principal Investigator: Kurt Stromberg

Section: General Biology

Lab/Branch: Laboratory of Biology , DCBD

Goal: Characterization of a representative animal type-C RNA virus in order to develop methods for detection of similar viral information in human tissues.

Approach: The experimental design has been to fractionate AMV into its component parts by two successive methods: firstly, by exposure of intact AMV to a nonionic surfactant which separates the viral "envelope" from the viral "core" and secondly, by exposure of the resulting viral cores to high concentrations of sodium chloride which releases the inner viral "nucleoid." The three components are separated by ultracentrifugation, and then characterized by electron microscopic, biochemical, and enzymatic methods. These procedures revealed a specific RNA methylase to be localized as a non-core constituent of AMV, and established the presence of transfer RNA in the core component, as well as the 60-70S RNA. The core contains 86% protein, 8% RNA, and 6% phospholipid as compared to 62% protein, 3% RNA, and 35% phospholipid in the virion of AMV. The core does not contain glycoprotein, or a major 22,000 M.W. polypeptide, which are found in intact preparations of AMV. Polypeptides of 91,000 and 64,000 M.W. appear to correspond to the two subunits in purified AMV DNA polymerase, and thus identifies which of the 20 proteins in AMV represents reverse transcriptase activity.

Progress: Recent work has focused on the method for selective release and partial purification of the DNA polymerase in the AMV core by exposure to sodium chloride (which rapidly achieves a greater than tenfold purification). The unique and important advantage of the procedure is that the DNA-polymerase-containing core is separated from possible non-viral contaminants and membrane components before release of the DNA polymerase by salt treatment. This procedure therefore may assist in the detection of viral particle-associated DNA polymerase activity in crude biological materials such as tumor extracts, samples of milk, etc.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Early detection of preclinical cancer in man represents a major objective of current cancer research. The possible association of type-C RNA tumor viruses in human cancer dictate that the structural, biochemical, and enzymatic characteristics of animal type-C viruses be determined as a prerequisite to detection of similar viral-like characteristics in human fluids and precancerous tissues.

Level of Effort: Professional Man-Years: Two

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3256

Title: Ultrastructure and Biology of Mammary Tumor Virus Host-Cell Interactions

Principal Investigator: Gilbert H. Smith

Section: General Biology

Lab/Branch: Laboratory of Biology

Goal: To elucidate the aspects of cell-virus interaction in mammary tumor virus (MTV) infection

Approach: Isolation and purification of intracytoplasmic A particles (putative MTV nucleoprotein cores) from primary and transplanted tumors. Purification of B particles (mature MTV virions) from primary mouse mammary tumors. Biochemical, immunologic and biologic analysis and comparison of these two types of particles in order to establish an ontological relationship between them.

Progress: Polyacrylamide gel electrophoretic analysis and comparison of A particle structural proteins with those of MTV was performed. The results were reported in a manuscript published in the Journal of Virology, April 1973. Immunologic comparison of purified type A and type B particles by the Ouchterlony technique indicate one or perhaps two cross-reacting antigens which showed immunologic identity between A and B particles. This observation was confirmed by the MTV group working at The Netherlands Cancer Institute in Amsterdam. Studies are in progress to identify and elucidate what antigens are cross-reacting between the two particles in order to rule out a possible common specie-specific antigen present in both isolates. Purified intracytoplasmic A particles were found to be non-infectious (in contrast to B particles), when inoculated into newborn BALB/c/He mice. A manuscript describing these observations is planned.

Significance for Cancer Research (NCP Objective 3 Approach 3)

The mouse mammary tumor virus problem has been and is being used as a relevant model for understanding human mammary neoplasia. Yet our knowledge of the intracellular events in MTV infection is essentially nil. These studies provide a base upon which to establish this very important information.

Level of Effort: Professional Man-Years: 5/8

Serial No. NCI-3257 SAME
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Factors influencing the growth and neoplastic conversion of mammalian cells in vitro.

Principal Investigator: W.G. Taylor

Other Investigators: V.J.Evans, K.K.Sanford, R.R.Gantt, M.J.Taylor, F.M.Price, R.A.Dworkin, A.Kerr, and T.P.Yang

Cooperating Units: R.W.Pumper, Department of Microbiology, University of Illinois at the Medical Center, Chicago, Ill.; and R.Parshad, Department of Pathology, Howard University School of Medicine, Washington, D.C.

Project Description:

1. Initiation of primary C3H/HeN embryo cell cultures in medium supplemented with peptone or peptone dialysate.
2. Use of peptone and peptone dialysate for the continuous growth of cell lines currently used for human vaccines.
3. Rapid adaptation of an established C3H/HeN cell line to chemically defined medium; A potential diagnostic tool?
4. Further biochemical analyses of peptones and peptone dialysates.

Objective No. 1: Previous studies had shown that Bacto-peptone (Difco) and peptic peptone (NBC) supported active proliferation of two established cell lines, and that Witte's peptone (Frd. Witte Co.) and tryptic digest of casein (Colab) promoted survival and, to a lesser degree, proliferation (Exp Cell Res 74: 275, 1972). The purpose of this study was to extend the use of these peptones to primary C3H/HeN cell cultures.

Methods Employed: Freshly explanted C3H/HeN mouse embryo cells were grown in medium NCTC 135 supplemented with either a peptone or peptone dialysate. A "negative control" consisted of primary cultures initiated in chemically defined medium NCTC 135; cells grown in NCTC 135 supplemented with 10% v/v FBS represented the "positive control." Tumorigenicity was evaluated by injecting the cultured cells into irradiated syngeneic hosts.

Major Findings: The only peptone which supported continued growth of these mouse cells was Witte's peptone used at a concentration of 1.5-3 mg/ml culture medium. Growth of cells in this medium was slow for about 20 culture generations; the subculture rate was approximately once each 14 days. Following this period of slow growth, the cells grew much faster and could be subcultured weekly. After one year in culture, cells were injected into homologous hosts

and produced transplantable tumors. Two other cell lines in medium NCTC 135 with Witte peptone have been carried in vitro for over one year but as yet cannot be subcultured at a regular interval.

Two cell lines were grown in medium NCTC 135 plus a Bacto-peptone dialysate (PD) for approximately 9 months before dying out; during this time the cultures were subcultured 8 times. One cell line in Medium NCTC 135 plus dialysate of peptic peptone has been carried in culture for over one year although the growth is very slow and the population is very small. After one year in culture this line is still only in the 7th culture generation.

Cultures initiated in CDM all died within two weeks; those initiated in medium NCTC 135 plus 10% v/v serum survived as rapidly proliferating cell lines.

Significance and Proposed Course:

Since the test cultures were planted from common cell pools, it can be said that peptones promote survival, and in one case active proliferation, when compared to the cultures grown in CDM. Surprisingly, the whole peptone was more efficacious than the dialysate. None were as efficacious, however, as whole serum. This may reflect: a) the inability of peptones or peptone dialysates to stabilize in vitro perturbations (eg., pH changes, redox imbalances, etc.), as compared with serum; b) a cytotoxic component in peptones which prevents efficient initiation (survival and growth) of primary mouse cultures; or c) the presence of a heretofore unidentified "vitamin-like" factor in serum but not peptone. Alternatively, a mitotic stimulant for continuous lines may be the antithesis of whatever is required for the development of a stable, normal diploid cell line: that is, active proliferation may be inconsistent with specialized function and survival in a primary or secondary cell culture.

Objective No. 2: The aim was to ascertain if cell lines currently used for the production of human vaccines could be adapted to continuous growth in serum-free peptone or peptone dialysate (PD) culture media. Biologic markers such as growth rate, chromosome analyses, isozyme analyses, and virus susceptibility were studied.

Methods Employed: Parent cultures of the monkey kidney (MK₂) and rabbit kidney (RK₂) lines, used for the Salk polio and rubella vaccines, respectively, were adapted to growth in serum-free medium in MEM + 10% v/v Bacto-peptone and a final dextrose concentration of 200 mg%. After a brief lag phase, the cells grew well, and the whole peptone could be replaced with peptone dialysate (PD). LDH and G6PD analyses were performed by the method of Montes de Oca, Macy and Shannon (Proc. Soc. Exptl. Biol. & Med. 136: 462-469, 1969). Karyological analyses were performed by Dr. Ram Parshad, Howard University, Washington, D. C. The early culture work and the virus susceptibility studies were performed by Dr. Robert W. Pumper, University of Illinois, Chicago, Ill.

Major Findings: Both cell lines were adapted to peptone supplemented culture medium with relative ease, and grew equally well in PD-supplemented medium. Best growth was observed in cultures continuously propagated in

Dulbecco's modified MEM (containing four times the concentration of amino acids and vitamins as in MEM) supplemented with 2.5 mg PD/ml, wherein saturation densities exceeded 2×10^5 cells/cm² for both cell lines. G6PD patterns were typical for both; the MK₂ derivative showed two LDH bands which migrated just ahead and behind the single band normally observed in monkey cells. The karyotypes were typical. These cell lines showed no loss of susceptibility for the appropriate challenge viruses.

Significance and Proposed Course: This study demonstrates that an autoclavable serum substitute can be used successfully to grow significant numbers of MK₂ or RK₂ cells without sacrificing their general biological characteristics or susceptibility to clinically significant viruses. The implications for vaccine production are obvious, particularly since the most successful supplement, PD, is a mixture of dialyzable components, and therefore, probably not immunogenic. The heat stability of PD precludes adventitious microbial contamination of cell cultures by viruses, bacteria, fungi, or bacteria phage, all of which can be introduced into culture by serum.

Future studies include: A) the extension of this observation to "normal" human cells; and B) the full evaluation of the spontaneous neoplastic conversion of such cells. (See next section).

Objective No. 3.: The aim was the rapid "adaptation" of an established non-neoplastic C3H/HeN line to growth in chemically defined medium (CDM). Since the initiation of primary cultures (i.e., of freshly explanted tissue) is more difficult in the absence of serum, a method for rapidly adapting cells to growth in CDM would allow efficient primary culture initiation in microbe-free, high quality sera, and yet mitigate against the necessity for serum throughout the life of the culture. The adaptation must be rapid so as to be feasible for a broad spectrum of uses and investigators.

Methods Employed: Parent cultures of a nonneoplastic mouse line, NCTC 5857, are subcultured in medium NCTC 135 + 10% v/v fetal bovine serum (FBS) (Flow Laboratories, Rockville, Md.) After 264 culture transfers in vitro, at which time the cell line was not known to be tumorigenic, the serum content was reduced to 1% v/v and whole Bacto-peptone introduced at 10% v/v. Subsequently, the serum level was reduced to 0.25% v/v, then removed completely. Cultures grown continuously in NCTC 135 + 10% v/v Bacto-peptone were readily adapted to completely chemically defined NCTC 135. Tumorigenesis was assayed in irradiated, syngeneic animals with an inoculum of 10^6 cells per animal.

Major Findings: In preliminary studies, an extended culture period in each experimental medium was used to dilute residual serum components. Emphasis, therefore, was placed on survival and growth rather than the rapidity of the adaptation or the tumorigenicity of the resulting cell population. Nevertheless, adaptation for medium supplemented with 10% v/v FBS to completely CDM required only 160 days when peptone was used to compensate for the reduction in serum. In a control study, H. A. Kerr and F. M. Price adapted the same parent line to CDM by gradually reducing the serum content, but this required a greater time period.

Morphologically, the parent cultures are epithelial-like, but the strains in the experimental media tend to be fibroblastic. Though no quantitative studies have been done, they tend to grow as well or slightly better in the serum-peptone supplemented media.

After 15-20 subcultures in CDM, the cell population (strain NCTC 8081) became more epithelial-like. Injection of these cells resulted in 1 mm tumors in less than two weeks.

Significance and Proposed Course: First, this culture regime may provide a method for establishing low passage level cell strains grown in little or no serum. The advantage would be the rapid removal of a myriad of heterologous natural "effectors" -- eg., bovine hormones, enzymes, etc., acting on non-bovine cells. This in turn may promote greater phenotypic and genotypic stability in vitro.

Secondly, in the absence of "tumorigenesis data on the parent NCTC 5857, it cannot be incontrovertibly stated that this culture regime per se promotes a cryptogenic or "spontaneous" neoplastic conversion in vitro. Equally plausible is the explanation that the regime selected for a neoplastic population present in the parent culture (NCTC 5857) at a level too low to be detected by injection into C3H/HeN mice. This premise is supported by the fact that one of five clones derived from NCTC 5857 was recently shown to be neoplastic. (K.K.Sanford, unpublished data). If it can be demonstrated that this culture regime selects for a neoplastic population, rather than inducing a neoplastic conversion, it will provide important clues for diagnostic application.

Future studies will address themselves to these questions, and extend the procedure to tumor tissue as well as human and other species of cells. By studying the biological and biochemical characteristics of these lines, we hope to uncover differential markers (eg., differences in methylases) and hence redesign the milieu to provide greater cell stability.

Objective No. 4: Though free of microbial contaminants by virtue of its heat stability, peptone(s) represents a mixture of components as equally diverse as serum. Continued efforts are in progress to characterize the biologically efficacious component(s) in peptone. The ultimate objective is the development of a fully autoclavable culture medium capable of supporting a broad spectrum of cells -- particularly human cells -- either in active proliferative growth, or in a non-mitotic state.

Methods Employed:

A. In Vitro analyses: Rabbit and mouse cells were used to demonstrate biological activity of the total, free, and bound amino acids observed in peptone dialysate (PD). PD is a 25x purified supplement derived from peptone by reverse dialysis. Equal cell numbers can be obtained with one quarter the amount, w/w, of material per ml culture medium.

B. Other: Trace metal and vitamin analyses were performed by emission spectrometry and microbial growth assays, respectively (WARF, Madison, Wisc.).

Purine and pyrimidine analyses were undertaken on high pressure liquid chromatography (HPLC) and performed by Drs. R. R. Gantt and M. J. Taylor. Nucleoside and nucleotide analyses were carried out by acid hydrolysis of PD at 70°, followed by reducing sugar analysis of the hydrolysate.

Major Findings: Suffice it to say that zinc and iron were present in PD, and that PD contains a relatively high concentration of niacin; biological studies have not yet been done to determine if these facts are significant. HPLC analyses of PD demonstrated that, contrary to published reports (BBRC, 45: 1431-1436) no principal hypoxanthine-like peak was observed, and though other bases were identified, independent reducing sugar analyses demonstrated that as nucleotides they constituted no more than 1% w/w of PD.

On a weight basis, inorganic ash and total amino acids represent about 3.5% and 93.5% of PD, respectively. Approximately 10% of most amino acids are "free" when untreated PD is chromatographed as a physiologic fluid. Hence, it is probable that the remaining amino acids are bound, presumably as short peptides. When mixtures of the total, free, and bound amino acids (as calculated from the amino acid analysis) were tested on rabbit heart (RH) cells, the only positive response was observed in RH cells continuously subcultured in NCTC 135. This result, in a medium with a diverse vitamin and cofactor content, supports the explanation that the biologic efficacy of PD is a synergistic or additive response to several components. This may be resolved when a mixture of vitamin is prepared from the microbial assay data and evaluated in our test culture.

Significance and Proposed Course: Peptones and peptone dialysate have been demonstrated to be useful for primary and continuous mouse cell lines and for two virus-susceptible "vaccine lines." The further resolution of the efficacious component(s) is, therefore, of practical importance as well as theoretical interest.

Future work includes the completion of analytical work in progress, the use of ion exchange and gel filtration chromatography, etc., for the further chemical purification of PD. Ultimately, a more suitable culture medium incorporating the efficacious component(s) is envisioned.

Publications:

1. Taylor, W.G., Price, F.M., Dworkin, R.A., Evans, V.J.: A simple device for planting replicate mammalian cell cultures. *In Vitro* 7: 295-299, 1972.
2. Taylor, W.G., Dworkin, R.A., Pumper, R.W., Evans, V.J.: Biological efficacy of several commercially available peptones for mammalian cells in culture. *Exp Cell Res* 74: 275-279, 1972.
3. Evans, V.J., and Taylor, W.G.: Replicate Culture Methods: Monolayer Cultures, *in* Methods and Applications of Tissue Culture, Eds. P.F.Kruse and M.K.Patterson, Academic Press, Inc., New York, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3257

Title: Factors influencing the growth and neoplastic conversion of cells in vitro.

Principal Investigator: W.G. Taylor
Section: Tissue Culture Section
Lab/Branch: Laboratory of Biology

Goal: Characterization of an autoclavable serum-substitute, peptone and peptone dialysate, efficacious for the growth and survival of several species of mammalian cells.

Approach

This program requires a synthetic and analytic research approach. Characterization of a mixture of natural products by biochemical methods must be accompanied by the synthetic addition of these compounds to tissue culture medium with an appropriate biologic assay. In addition, the evaluation of a metabolite(s)' efficacy may be influenced profoundly by other parameters -- eg., variations in pH or pO_2 -- and also cellular requirements. Thus, the synthesis of data into a useful culture medium requires extensive experimental evaluation.

Progress:

During the past year, the following were dropped as biologically efficacious components of this serum substitute: most trace metals, fat soluble vitamins, mixtures of the common amino acids, nucleosides and nucleotides. Under evaluation is the specific role of zinc and iron, and the effect of water-soluble vitamins. The most likely candidate as an active factor are dialyzable peptides.

Significance for Cancer Research (NCP Objective 2 Approach 4)

The development of any cancer vaccines, the use of tissue culture as a diagnostic tool, and the elimination of serum (a source of adventitious contamination of cultures by viruses, bacteria, etc.) from research tools requires: an autoclavable substitute and a better understanding of in vitro cellular requirements.

Level of Effort: Professional Man-Years: 1/2 to 2/3 year

Serial No. NCI-3259

1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Host genome-viral relationship in mammary tumor development in mice

Principal Investigators: G. Vlahakis and W. E. Heston

Other Investigators: Gilbert H. Smith

Man Years:

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description: Objective 1. Approach 4

Objectives: To study the relationship between the viable yellow gene (A^{vy}) and the viral influence in the development of mammary tumors.

Methods: In previous observations it had been emphasized that the viable yellow gene (A^{vy}) which characterizes strain C3H- A^{vy} fB probably plays an important role in the development of mammary tumors in the strain. One of the possible actions of the A^{vy} gene may be to enhance the usually poor oncogenic ability of NIV (nodule inducing virus) which strain C3H- A^{vy} fB harbors. Another possibility may be that the A^{vy} gene is acting in some other way; for example, on the mammary gland itself, to account for the increased susceptibility to tumor development in this gland. Thirdly, the NIV of strain C3H- A^{vy} fB may represent a more potent virus.

Accordingly, strain C3H- A^{vy} fB which has a 90% spontaneous mammary tumor incidence, in the absence of the milk borne mammary tumor virus (MTV), was outcrossed to the agouti strain C3H/StWi, in which mammary tumors have ceased to develop, and in which no MTV or NIV has been demonstrated. The F₁'s were then backcrossed to C3H/StWi to produce two classes of animals: viable yellow (A^{vy} A) and agouti (AA). Both groups will be expected to have NIV that is passed vertically at the time of conception by the C3H- A^{vy} fB parent. The NIV is transmitted equally well by either the female or male parent of strain C3H- A^{vy} fB.

Results: There were five (71.4%) strain C3H-A^{Vy}fB females with mammary tumors in a total of seven used in outcrosses to strain C3H/StWi males to make F₁'s. The average tumor age was 16 months. The two females without mammary tumors were necropsied at an average of 16.5 months. The mammary tumors were those typical of strain C3H-A^{Vy}fB females.

There were also three females with hepatomas occurring at 17 months average age of death.

From a total of 38 (C3H-A^{Vy}fB x C3H/StWi)F₁A^{Vy}A females used to produce both classes of backcrosses, 11(28.9%) had mammary tumors occurring at an average of 19.3 months. The average age of death for those without mammary tumors was 18.8 months. One of the tumors is being examined by Dr. Gilbert Smith for the presence of B particles which would represent NIV.

Since the A^{Vy} gene has been shown to increase the occurrence of hepatomas, approximately 90% of the A^{Vy}A-F₁ females had these tumors at 19.5 months average age of death. The other principal neoplasm was pulmonary tumor which occurred in 34% of the females.

The 89 A^{Vy}A(C3H-A^{Vy}fB x C3H/StWi)C3H/StWi BC females had an average of 1.4 litters, compared with 2 litters for the 104 AA(C3H-A^{Vy}fB x C3H/StWi)C3H/StWiBC females. There were 7 (7.9%) mammary tumors in the A^{Vy}A-BC females arising at an average age of 17.1 months, compared with 4 (3.8%) mammary tumors in the AA-BC females arising at an average of 20 months of age. One adenocarcinoma of the mammary gland from an A^{Vy}A-BC is being examined by electron microscopy, for B particles. One other adenocarcinoma of the mammary gland from an A^{Vy}A-BC as well as one adenoacanthoma of the mammary gland from an AA-BC female are also being examined for B particles.

The mammary tumors in the A^{Vy}A-F₁, A^{Vy}A-BC and AA-BC females were similar in morphology to those obtained in the hybrid females between strains C3H-A^{Vy}fB and BALB/c. Like strain C3H/StWi, strain BALB/c does not have MTV or NIV.

Many of the A^{Vy}A-BC females were in poor condition from hepatomas and had to be necropsied. Approximately 90% of them had hepatomas arising at 18.8 months average age of death, compared with only 25% for the AA-BC females arising at 24.4 months average age of death.

The other principal neoplasm in both backcross groups was pulmonary tumor. There were 18(20.2%)A^{Vy}A females with pulmonary tumors occurring at 19.7 months average age, compared with 22 (21.1%) AA females occurring at 24.6 months average age. The higher number of pulmonary tumors in the A^{Vy}A-F₁ and both backcross groups of females compared to strains

C3H-A^{Vy}fB and C3H/StWi, can be attributed to the average longer life-span in all the three groups.

While there had not been a large number of mammary tumors in the A^{Vy}A-F₁ and both backcross groups of females, those tumors that did occur indicated that those females with the A^{Vy} gene were more susceptible than the AA females. There were a few more tumors and they arose on the average earlier in the A^{Vy}A-F₁ and A^{Vy}A-BC females than in the AA-BC females. Because of the increased susceptibility of females with the A^{Vy} gene to the development of hepatomas, many had to be necropsied and may account in part for the lower numbers of mammary tumors seen in those groups. Approximately 90% of A^{Vy}A-F₁ and 87% of the A^{Vy}A-BC females had hepatomas compared with only 25% for the AA-BC females.

The significant result, however, that there were low numbers of mammary tumors in all the groups of females despite crosses essentially between "C3H" strains, suggests that genes from strain C3H/StWi inhibit mammary tumor development, possibly by selecting against NIV. It is interesting that in 1966 Dr. Gilbert Smith reported a 3% mammary tumor incidence for strain C3H/StWi, which is similar to the 7.9% and 3.8% obtained in this study, for the A^{Vy}A-BC and AA-BC females, respectively.

We are still attempting to develop a strain BALB/c with NIV from strain C3H-A^{Vy}fB, and a strain BALB/c with NIV from the agouti strain C3HfB. This will make it possible to compare the NIV's from the two strains in genetically similar hosts, in relation to mammary tumorigenesis. The NIV in strain C3H-A^{Vy}fB (with 90% mammary tumors) as compared with NIV in the similar strain C3HfB (with 40% mammary tumors), may represent a more potent NIV with a higher tumorigenic capacity. If on the other hand, the A^{Vy} gene enhances the usually poor oncogenic ability of NIV, there should be no difference in mammary tumors between strains BALB/c with C3H-A^{Vy}fB NIV and BALB/c with C3HfB NIV.

As a test of whether strain C3H/StWi transmits any agent in the milk, as ~~M~~TV is transmitted, the fostered strain BALB/cfC3H/StWi has now been developed. Of the females necropsied thus far, there has been no increase in mammary tumors above what is normally seen in strain BALB/c.

Proposed Course of Experiment:

The study is being concluded and the results are being analyzed for future publication.

Publications:

Heston, W. E., Vlahakis, G., and Smith, G. H.: Mammary gland tumors in substrains C57BL/M and C57BL/He mice. J. Nat. Cancer Inst. 49: 805-812, 1972.

Woods, Mark W., and Vlahakis, G.: Anaerobic glycolysis in spontaneous and transplanted liver tumors of mice. J. Nat. Cancer Inst. 50: _____. 1973.

Heston, W. E., Vlahakis, G., and Desmukes, B.: Effects of the anti-fertility drug Enovid in five strains of mice with particular regard to carcinogenesis. J. Nat. Cancer Inst. 51: _____, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3259

Title: Host Genome-Viral Relationship in Mammary Tumor Development in Mice

Principal Investigator: G. Vlahakis, W. E. Heston, and G. H. Smith

Section: General Biology

Lab/Branch: Laboratory of Biology

Goal: To study the relationship between the viable yellow gene (A^{vy}) and the viral influence in the development of mammary tumors

Approach: Strain C3H- A^{vy} fB which has a 90% spontaneous mammary tumor incidence, in the absence of the milk borne mammary tumor virus (MTV), was outcrossed to the agouti strain C3H/StWi, in which mammary tumors have ceased to develop, and in which no MTV has been demonstrated. The F_1 's were then backcrossed to C3H/StWi to produce two classes of animals: viable yellow ($A^{vy}A$) and agouti (AA). Both groups will be expected to have the variant mammary tumor virus, NIV, that is passed vertically at the time of conception by the C3H- A^{vy} fB parent. The NIV of strain C3H- A^{vy} fB is transmitted by the male parent as readily as by the female parent.

Progress: While there had not been a large number of mammary tumors in the $A^{vy}A-F_1$ and both backcross groups of females, those tumors that did occur indicated that those females with the A^{vy} gene were more susceptible than the AA females. There were a few more tumors and they arose on the average earlier in the $A^{vy}A-F_1$ and $A^{vy}A-BC$ females than in the AA-BC females. Because of the increased susceptibility of females with the A^{vy} gene to the development of hepatomas, 90% of $A^{vy}A-F_1$, and 87% of the $A^{vy}A-BC$ females had hepatomas compared with only 25% for the AA-BC females. The low numbers of mammary tumors in all groups despite crosses essentially between "C3H" strains, suggests that genes from strain C3H/StWi inhibit mammary tumor development possibly by selecting against NIV.

Significance for Cancer Research (NCP Objective 1 Approach 4)

The A^{vy} gene of strain C3H- A^{vy} fB is an example of the role of genes in tumorigenesis. Tumors result from the interaction of a suitable genotype with hormones and viral agents.

Level of Effort: Professional Man-Years: 1/2

Serial No. NCI-3260

1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Studies on the Mammary Tumor Virus in Resistant Strain C57BL Mice

Principal Investigator: W. E. Heston

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description: Objective 1 Approach 4

Objective: The aim of this study is to ascertain if there is a mammary tumor virus in this genetically resistant strain and to see if this strain can propagate the mammary tumor virus introduced from susceptible strains.

Methods: This study is approached through the development and study of foster-nursed lines. Two lines of fostered C57BL mice have been started, a C57BLfC3H line started from a litter of C57BL mice foster-nursed on a strain C3H female with the MTV, and a C57BLfRIII line started from a litter of C57BL mice foster-nursed on a strain RIII female with the MTV. The two lines are being maintained by brother sister mating to see if the MTV will survive in the strain or if that from the RIII strain will survive whereas that from C3H will not for which there already is some evidence.

A third line BALB/cfB has been started from a litter of very susceptible BALB/c mice without MTV foster-nursed on C57BL. This line will likewise be continued to see if there is a repressed MTV in C57BL which can replicate when introduced into the BALB/c.

Results: The lines are just getting started and thus far no tumors have appeared in any of them. When they occur we would hope to examine them by EM for B viral particles or by immunofluorescence for MTV antigen.

Significance of Program to the Institute:

This is a part of the whole mammary tumor program of the laboratory and particularly the genetic transmission of the mammary tumor virus. If there is a provirus that is inherited as a segment of the chromosome do all mice have it as has been postulated? In this regard it is very important to know if C57BL has the genes for the replication of at least some lines of the virus and even more important to know if C57BL has a repressed virus, that is, has the provirus. This is important in regard to mammary cancer in women for it appears that the genetic transmission of the mammary tumor virus in the mouse is the more appropriate model for the identification of a virus in human breast cancer. As a part of this whole program we have a contract supporting the program of the Netherlands Cancer Institute in this area and the principal investigator of the project reported here is the Project Officer of that contract.

Proposed Course:

The whole program in this area will be pursued to the identification through hybridization studies of a segregating locus that controls the postulated provirus and the location through linkage studies of this locus on the chromosome. This particular facet of the program will be pursued to answering the question of whether this resistant strain has the mammary tumor virus, or its provirus, and whether it is capable of replicating any line of the MTV.

Publications:

Heston, W. E., Vlahakis, G. and Smith, G. H.: Mammary gland tumors in substrains C57BL/M and C57BL/He mice. J. Nat. Cancer Inst. 49: 805-812, 1972.

Heston, W. E.: Genetics of mammary cancer , Chapter III, In Methods in Cancer Research, Volume VII (Harris Busch, ed.) Academic Press, Inc., New York, pp 115-129, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3260

Title: Studies on the Mammary Tumor Virus in Resistant Strain C57BL Mice

Principal Investigator: W. E. Heston

Section: General Biology

Lab/Branch: Laboratory of Biology

Goal: To ascertain whether there is a mammary tumor virus or provirus in the genetically resistant strain C57BL and to see if this strain can replicate the MTV or any line of MTV.

Approach:

The approach is through the development of fostered lines. One line, C57BLfC3H, has been started from a litter of C57BL foster-nursed on C3H and another, C57BLfRIII, has been started from a litter of C57BL foster-nursed on RIII. The two lines will be maintained by brother and sister matings to see if both replicate the MTV received from the foster mothers or if the RIII MTV is replicated whereas the C3H MTV is not as has been postulated. A third line, BALB/cfB, has been started from a susceptible BALB/c litter foster-nursed on C57BL to see if it receives from the C57BL a line of MTV which has been postulated.

Progress:

The lines are set up but thus far no mammary tumors have appeared. When and if tumors appear they will be examined with EM for the presence of B particles and immunofluorescence for MTV antigen.

Significance for Cancer Research (NCP Objective 1 Approach 4)

This is a part of the whole program of the factors involved in mammary tumorigenesis in mice and particularly the genetic transmission of the mammary tumor virus. If there are human tumor viruses they are probably transmitted genetically and the genetically transmitted tumor viruses in mice such as some lines of MTV will be appropriate models for identification of the human tumor virus.

Level of Effort: Professional Man-Years: 1/4 per year

Serial No. NCI-3262 SAME
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972, through June 30, 1973

Project Title: The development of a homeostatic cell culture system and/or growth promoting system for cells in vitro.

Principal Investigators: W.G. Taylor and V.J. Evans

Other Investigators: K.K. Sanford and R.R. Gantt

Cooperating Units: V.P. Perry, C. Kroner, American Foundation for Biological Research, Biomedical Research Division, Rockville, Md.

Project Description:

Objective:

Current cell culture media and techniques subject mammalian cells in vitro to a myriad of physical and chemical variations. This constant physio-chemical perturbation undoubtedly influences growth, and at the very least, limits maximum population potential. These fluctuations may have bearing on the spontaneous malignant transformation that occurs in cells explanted in vitro.

Our immediate objective is the development of a cell culture system wherein nutritional and physio-chemical variation can be controlled within specified limits. Long-term goals include the elucidation of physio-chemical requirements for phenotypic and genotypic stability and ultimately the cause of the "spontaneous" neoplastic conversion. We view these objectives to be of immediate importance to the development of better human vaccines and in vitro cancer diagnostic tools.

Methods and Results:

During the past year, design and operational improvements were made in the pulsatile perfusion system. Earlier studies were limited to a single T-15 culture vessel; currently up to ten culture flasks can be perfused simultaneously and semi-independently. Instrumentation for automatically monitoring pH, pO_2 , pCO_2 , and flow rate have been integrated into the system, and additional apparatus received in recent weeks will allow us to miniaturize the monitoring capabilities while improving the sensitivity attainable.

In addition to a human diploid skin strain which was maintained as a confluent, essentially non-mitotic culture for 36 days, several studies involving actively proliferating human, diploid Wi-38 cells have demonstrated the system's usefulness. Approximately, a three-fold increase was observed in a

5-7 day incubation period. Further biologic evaluation of this system was hampered, however, by a shortage of hand-crafted glass components.

Proposed Course: Planned are further studies to 1) validate the instrument's efficacy for mammalian cell culture; 2) integrate the monitoring capabilities with instrumentation for controlling physiologic variation, thus converting a perfusion system to a homeostatic culture system; 3) extend the use of the pulsatile perfusion system to a quantitative analysis of the physio-chemical requirements of normal diploid, particularly human cells, and to the biology of the neoplastic transformation (conversion). Germane to the last will be a consideration of biochemical, cytologic, and karyologic alterations in vitro as influenced by the milieu.

Significance: The major significance is to provide a system with minimum trauma (physiologic, biologic, and biophysical) to the cells in culture. Therein we can monitor, control, and study a number of factors that could influence the malignant conversion of cells in vitro and thus, hopefully, obtain by control of these factors a normal or at least non-malignant cell population. In addition, there should be a number of ancillary benefits from the use of such a pulsatile perfusion system. We anticipate that mass amounts of cells might be grown in this system -- such cells as lymphocytoblasts, cells producing biologically active components not yet synthesized in the laboratory at all or only in minute amounts, namely certain glycoproteins, hormone-like substances, etc. Virus producing cells also could be grown in large amounts for vaccine production in such a system. The system is so designed that it is unusually non-traumatic to cells and might well be used for growth of interferon producing cells in the absence of serum or at the very least small molecule protein additives. Presumably a greater number of cells grown under ideal metabolic conditions would produce greater quantities of this antiviral agent. Recalling that mouse liver epithelial cells from early studies in this laboratory where cells were subjected to daily fluid renewal produced as many as 30×10^6 cells/ml of nutrient fluid in routine suspension cultures, we might well expect yields in this system the equal or higher without the need to resort to expensive hand labor. We visualize the system as ultimately being completely amenable to automation.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3262

Title: The development of a homeostatic cell culture system and/or growth promoting system for cells in vitro.

Principal Investigator: W.G. Taylor and V.J. Evans; V.P. Perry, C. Kroner.

Section: Tissue Culture Section

Lab/Branch: Laboratory of Biology

Goal: Development of a cell culture perfusion system: 1) to elucidate the physio-chemical requirements of mammalian cells and provide a homeostatic environment by controlling variation in the milieu; 2) to evaluate the influence of these parameters in the in vitro neoplastic conversion (transformation).
Approach

The pulsatile pump perfusion system operates similarly to the mammalian cardiovascular system, using a "systolic-diastolic" pumping mechanism. During FY-1972, pH, pCO_2 , and pO_2 automatic monitoring devices were installed and pilot growth studies done. With this preliminary data, we will establish limits for purposes of stabilizing the culture milieu; through this we hope to ascertain the influence of in vitro perturbations upon normal growth, function, and the neoplastic conversion.

Progress:

Progress has been slow; support personnel with proven biological and bioengineering expertise are rare and their time and talents are in demand for programs of lesser complexity and greater financial return. We hope contract funds of modest proportion (\$50,000-100,000) will be available in the near future.

Significance for Cancer Research (NCP Objective 3 Approach 2)

It would seem that a homeostatic culture system, as described, would be essential to an incontrovertible analysis of neoplastic transformation. Other objectives, approaches, approach elements, and project areas this project supports are: 2.4.3.8, 5.5, 6.1, 6.2, 6.3, and 6.6, as its success will supply a need in many areas.

Level of Effort: Professional Man-Years: 1/4 yr.

Serial No. NCI-3263 NEW
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Developmental research on new methods for the cultivation of very large numbers of human and other mammalian cells in vitro.

Principal Investigator: V. J. Evans

Other Investigators: W.G.Taylor, R.F.Camaller, F.M.Price, T.P.Yang, and C.H.Fox (Guest Worker)

Cooperating Units: Biomedical Research Institute of American Foundation for Biological Research, Rockville, Md. 20852

Man Years: See Serial No. NCI-3211

Project Description: Studies to develop large populations under controlled physiologic conditions of both mouse and human cells in surface substrate cultures of so-called "fixed tissue" cells.

Objectives: To investigate new and innovative methods for the cultivation of large amounts of cells requiring attachment to a solid substrate, and to adapt these methods to the cultivation of human and other mammalian cells. Using these methods we propose to:

- (a) Develop new methods of delivering physiological necessities such as nutrients, gases, and replenished or restored media to cultures.
- (b) Study the possibilities of cultivating cells such as "finite life" human fibroblast cultures and freshly isolated cells and human tumor cells.
- (c) Study the possibilities of growing cells in defined media in massive cell cultures.
- (d) Cause the malignant transformation of cultures by varying the parameters affecting growth of cells in large cultures.
- (e) Establish the economies of increased scale production of living cells under novel cultural conditions without concomitant increase in working space or with only a proportionate increase in the amounts of media and culture space needed.

Introduction:

Mammalian cells have been cultivated in vitro for many years. These cultures fall into two general types: cells that require attachment to a surface ("fixed tissue cells"), or cells that are capable of growing in suspension culture. Cells that require a solid substrate for growth include nearly all freshly isolated cultures, as well as most of the "non-transformed" cells, those that have not been found to produce tumors when injected into isologous animals.

The current methods of supporting growth of cells in culture provide for a substrate of glass or plastic over which is layered a liquid medium. The cell inoculum is at a density ranging from 10 cells per square cm to 10,000 or more per square cm of available surface area in the flask. This initial inoculum grows, over some period, to a maximum density of between 10,000 and 500,000 cells per square cm. The actual numbers of cells produced depends on a number of ill-defined factors that include the methods used to replenish the medium, the type of medium, the nutritional requirements of the cells, etc

The medium is an important factor in this type of culture. Most media used in tissue cultures contain a number of readily exhaustible nutrients, a number of components that may or may not be required, a buffer system that frequently is of the carbonate/bicarbonate/carbonic acid/CO₂ type which requires a gaseous equilibrium as well as a liquid phase adjustment, and a supplement of a vital fluid derived from a mammal, such as fetal calf serum, horse serum, or other undefined composites of factors acting in still unknown ways to support growth and possibly membrane integrity.

These media must be replaced at various time intervals, so that the yield of cells per ml of medium actually used is on the order of 1×10^6 cells per ml of medium or less for a typical fibroblast culture. In addition, there is a quantitative difference between cultures in which the medium has been subject to frequent replacement and those changed less frequently.

The labor involved in frequent changes of culture medium, the added cost of inadvertent contamination, the necessity of rigid enforcement of costly aseptic conditions, and the investment in exotic serum components make the cultivation of cells an expensive and labor intensive process.

To further complicate matters, cells cultivated for long periods often undergo a "malignant transformation" after having been in vitro for some period. This event, though studied for many years, is of not yet determined etiology and is difficult to define in physiological or biochemical terms, but renders cells unusable for studies of typical "normal" tissue components and currently absolutely eliminates their utility in preparation of biological products for therapeutic use.

Methods and Findings:

We first intend to develop the recent invention of a disposable plastic receptacle intended for the large-scale cultivation of cells. This device, marketed by American agents for the National Research Development Corp. Ltd., a British firm, consists of a long rectangle of plastic film (Melinex^R) that has been rolled to form a cylinder. The rolls of film are separated by corrugated strips that maintain a constant distance between layers of film. The roll is contained in a plastic cylinder that is formed in two pieces to form a canister joined at the middle by a secret process. This outer canister has three screw cap openings at one end, thus allowing the introduction of a gas through the center of the canister by a sparging tube that extends to the bottom of the canister. The other two openings allow media to be added or withdrawn, the inoculation of the canister with cells, venting of the sparged gas, or other necessary services. The device is sterilized by another secret

process. The proposed manufacturer's cost is \$15.00, a considerable price. The unit, as with many plastic products, is quite fragile.

The surface area to be covered by the cell sheet is approximately 8000 square cm and the device is designed to be charged with 1600 ml (2000ml actual capacity) of media. This provides a media to surface ratio of 0.2 ml per sq. cm. The ratio usually employed in cultivation in the conventional T-series flask is on the order of 0.2 to 0.5 ml per sq.cm. According to the patent the device may be employed in a number of ways, but is in general to be incubated standing as a vertical cylinder with relatively small amounts of gas bubbled through the medium (0.1 to 0.3 liters per minute). The inventors claim to have obtained an 11.5 fold increase in five days, or an increase of 9,200,000 cells per sq.cm. above the inoculum. Unfortunately, the cells used by the inventors are believed by many to be a "transformed" line (BHK Cl3) and may therefore grow more readily than "normal" cells. In addition, this increase, when considered on the basis of cells produced per ml of medium is not so favorable as would appear. In the ordinary T15 culture flask it is not uncommon to realize a net yield of 650,000 cells per ml of medium when three changes of medium are made, and usually the yield is much higher. The best yield reported from the Sterilin device is about 460,000 cells per ml of medium, net. On a cost basis, the plastic apparatus commercially available may have at best only a marginal advantage insofar as the basic investment in apparatus is concerned.

Claims for production of primary cells from mouse embryo preparations (doubtless maximal in a patent application) are 10^9 cells produced from an unstated inoculum in 2 liters of medium, which in actual practical use cannot be introduced into the canister. In any case, the design as described is capable of producing only one order of magnitude increase in cell number over conventional methods.

Our experience with this device has indicated that, while promising, the design and materials leave much to be desired. In several experiments we have proceeded as follows: The device was charged with medium and cells were introduced as a suspension through the orifice and the cylinder was allowed to rotate slowly on its side on a laboratory roller device for four hours to allow the cells to attach. The cylinder was then placed upright and gassed at a rate of 0.5 liters of 5 or 10% CO_2 in air per minute in intermittent (10 sec) bursts (10 sec off). In medium requiring serum we have added standard defoaming substances (silicones) as recommended by the suppliers of the device. The defoamer chosen by us was antifoam C emulsion (Dow Corning), which is a good grade agent approved by FDA. By experiment it was learned that the antifoamant cannot be autoclaved more than once or twice without losing effectiveness.

Wi-38 human finite life fibroblasts that will not grow in suspension have been planted by us into the Sterilin plastic canister type receptacle. At the end of 4 days the entire uncoiled plastic sheet was covered over its entire length and width to confluence on the concave side; since the sheet was confluent, the number of cells in division per field was limited. Only a very few cells were found on the convex surface and these were in poor condition. Some patches were found on the flat-bottom surface of the canister, probably

emanating from cells that fell from the suspension when the canister was placed in upright position for intermittent gassing. It is interesting that only the concave surface of the plastic film was confluent. Only 4000 sq cm of coil surface was effectively utilized by this strain of human cells rather than the 8000 sq cm potentially available in both sides of the plastic coil. It is not yet determined whether the failure of cells to attach to the convex surface is a function of this surface being untreated with the "corona discharge" stated by the manufacturer as necessary for the surfacing of MelinexTM to support cell attachment. This is a point that can be answered by studies in progress on better plastic coils, better cleaned and processed. Also still to be determined is the minimum inoculum population that can be used to charge the vessel. The total achievable population on 4000 sq cm under current medium volume and gassing conditions of this design Sterilin canister probably cannot exceed that obtained from 53-54 T75 flasks at confluency. However, this is a sizeable number considering labor saved. Alteration by perfusion methods of gassing and medium exchange has the potential of leading to layered cell growth and therefore increased populations. All this is under exploration with new vessel designs arising from evaluation of the inadequacies of the original Sterilin canister.

So far, the greatest increase in growth has been far less than that claimed, a result not unexpected. In several experiments with mouse cells, growth has been difficult to measure because of difficulty in harvesting cells, but certainly does not exceed increases in biomass much over a factor of 3 to 4. In addition, the cells have been accompanied by considerable debris and dead cells in the case of the mouse cells, but less so with human cells.

An attempt was made to grow mouse cells in chemically defined media in this device without added serum,--a routine practice in our laboratory and one that has resulted in consistent production of 1×10^6 cells per ml of medium in T-flasks. In the Sterilin device there was only a modest increase of several fold in biomass over the size of the inoculum. However, problems were encountered in harvesting cells.

In other experiments the charged device with 2 cell types was allowed to roll on a roller without addition of a gas mixture. Little cell growth was obtained. We do not believe that the Sterilin device of National Research and Development Corp. is a total failure, but from our experience we believe that a drastic change in design and operation is mandatory before this type of device will achieve its full potential if cells are to be grown in a rotating system rather than plating. It is expected that constant gassing will improve the yield.

The principal difficulties in the mass cultivation of attached mammalian cells are due to three problems we now believe to be remediable. The first of these is a lack of understanding concerning the metabolic needs of attached cells confined to a high density population. The second is an absence of knowledge concerning the respiratory requirements of such cells, particularly as regards gas tension in the medium. Finally, there is some confusion about medium depletion and renewal.

Each of these variables is amenable to study and correction using technology now available to us. Recently we have designed a device similar to the Sterilin in some characteristics but markedly improved in others. The first of our adaptations substitutes a glass outer vessel. The material used in the coil is an improved heat stable plastic. The glass modification is provided with a nylon or teflon cap that can be fitted with a swivel that allows the vessel to be laid on its side while gas is added through the cap and the bottle is being rotated. Another modification of this design allows gas to be used to agitate the culture fluid through diffusers built into the bottom of the coil thus eliminating the danger of dead spots, if any, in the film layers. Dead spots due to other causes are subject to evaluation.

Departing from the principles of the Sterilin device, we are considering some new and innovative devices that are specifically designed to agitate constantly and aerate the medium. One of these consists of a cylindrical helix made of hollow plastic or other material suitable for attachment of cells. On the inside of the hollow helix, cells will be allowed to attach to the walls of the enclosure, and the vessel will be rotated while gas is added through a swivel valve at the center of the helix. The coil is turned so that the gas in the center of the coil is carried to the outside of the helix where it is discharged through another swivel.

Another model consists of a glass mandrel winding much like that in condensers in a distillation apparatus. Cells will be allowed to coat the interior of the coil while medium and gas will be added either constantly or intermittently. It is conceivable that a capillary device could be constructed that would provide a very large surface compared to a conventional cylinder.

Many other designs are possible and we expect to have access to them should our present designs prove unsatisfactory. It is certain that the type of vessel that we will use is not a limiting factor to the success of this work.

The major experimental difficulty we expect will be in design of a suitable medium for continuous cultivation of large quantities of cells. Recently we have constructed a modern version of the Lindberg pump constructed entirely of non-toxic materials. This pump can be used to perfuse any type of culture vessel on an intermittent (pulse) or continuous basis. Two added advantages of this pump are that it allows for a reservoir of medium and may be programmed for the exposure of the medium to gas mixtures while remaining sterile. Sample ports allow continuous monitoring of variables in the medium such as pO_2 , pCO_2 , and pH. This device will allow extreme versatility in designing media because it will be possible to monitor such variables as uptake of essential nutrients, concentration of waste products, and rate studies of depletion with increase in cell numbers, without significantly reducing the volume of the culture medium, due to the large reservoir sump.

Should present media formulation prove unsatisfactory, we believe that we will be able to alter concentrations during the progress of culture growth so that it will be possible to correct deficiencies before they become critical. We are particularly ambitious about the use of defined media in this system and new formulations are under constant design and test (see Serial No. NCI-3212, project 1.)

Another recent development that we would like to investigate is the set of possibilities presented by the advent of perfluorinated carbon compounds that are very active carriers of gas, particularly oxygen. When large amounts of cells are cultivated in a very small space, oxygen needs may exceed the capacity of the medium to transport oxygen and the cells become partially anaerobic. The effects of this situation have not been studied in large cultures, and we believe that it may seriously limit the usefulness of many culture devices and may be detrimental to the proper development of the cells. These perfluorinated carbon compounds may be useful in oxygen transport under this situation.

We believe that all of the necessary materials and equipment (possibly excluding a particular type of standard centrifuge) for development of methods for the large-scale production of cells are now available, and our preliminary experiments have made us quite certain that we have adequate facilities for such developmental research. Human resources are also ideal for the type of research we are suggesting and we can see no serious impediment that will prevent us from realizing the goals we have suggested here.

Significance and Summary:

The cultivation of large quantities of eukaryotic cells is fundamental to a number of avenues of attack on the cancer problem. The principal difficulty in obtaining the requisite amounts of living cells is due to the fact that cells, either freshly isolated from tissue or in culture but which have not undergone a malignant change (allowing them to produce tumors in normal animals), must be attached to a surface so that they may grow. The reason for this phenomenon is largely unknown despite having been under intensive study for at least four decades. While the theoretical possibilities of this question are intriguing, the pressing need for vast amounts of living cells that now exists and which is sure to increase in the immediate future has directed us to the research outlined here.

The disposable plastic cylinder with its coiled growing surface presents certain advantages in that it allows us to observe the characteristics of cell populations confined to small spaces, but we believe that it in no way represents a final solution to the problems of cultivation of homogeneous masses of cells. On the other hand, we believe that with developmental experimentation it will be possible to perfect a simple system of cultivation that will be economical of space, culture media, labor, time, and capital investment.

The points that require the greatest immediate effort concern methods of providing efficient gas transfer to the liquid medium, of insuring replenishment or sufficient addition of metabolites, of determining methods for the most efficient attachment rates of cells to the substrate, and agitation of the liquid contents of the vessel.

Problems that are now subject to immediate solution are those of the development of rotary perfusion caps, selection of autoclave-stable plastic growth surfaces, antifoam agents, and gassing mixtures. In coming weeks we believe that we will have information allowing transition from cultures that are numbered in the millions of cells to methods that will realize cells produced in gram and decigram quantities.

Development of mass cultivation methods for human cells with finite life, of freshly explanted cells, and of the many other types of malignant and non-malignant cells is an event expectantly awaited by biochemists, producers of biologicals and vaccines, and those in basic research. We believe that the possibility is within immediate reach and that this project will be a major advance in that direction.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3263

Title: Development research on new methods for the cultivation of very large numbers of human and other mammalian cells in vitro.

Principal Investigator: V.J.Evans; Co-investigator, W.G.Taylor

Section: Tissue Culture Section

Lab/Branch: Laboratory of Biology, NCI

Goal: To investigate new and innovative methods for cultivating mass amounts of cells demanding solid substrate, adapt these methods for human normal and malignant and other mammalian cells for general use, and ultimately control Approach / of normal and malignant cells.

(a) Develop new methods of delivering physiological necessities such as nutrients, gases, and replenished or restored media to cultures in innovated receptacles.

(b) Study the possibilities of cultivating cells such as "finite life" human fibroblast cultures and freshly isolated cells and human tumor cells in new type vessels.

(c) Study the possibilities of growing cells in defined media in massive cell cultures.

(d) Cause and hopefully control the malignant transformation of cultures by varying the parameters affecting growth of cells in large cultures.

(e) Establish the economies of increased scale production of living cells under novel cultural conditions without concomitant increase in working space or with only a proportionate increase in the amounts of media and culture space needed.

Progress: A new commercial device with a plastic coil for cell attachment has merit for growing cells demanding a substrate such a finite life human cells, freshly explanted non-malignant cells, and human tumor cells requiring surface. Large masses of finite life cells and mouse cells have been grown by us on the coil. Most importantly, the defects of the commercial device have been discovered; many are amenable to corrective measures, some already being carried out with new instrumentation designed and/or conceived in our laboratory. Among these are 1) autoclavable plastic coils, 2) devices for coiling the plastic, 3) methods for processing plastic to increase available surface for cells, 4) swivel caps for perfusing gases and nutrient fluids to increase yield of cells and reduce media usage, 5) introduction of FDA acceptable antifoamants for protein medium.

Significance for Cancer Research (NCP Objective 3 Approach 1,2) The cultivation of large quantities of eukaryotic cells is fundamental to a number of avenues of attack on the cancer problem. Development of mass cultivation methods for human cells with finite life, freshly explanted cells, and many other types of malignant and non-malignant cells is an event expectantly awaited by biochemists, immunologists, and producers of biologicals and vaccines, if and when required for cancer control. By "programming" the physiological necessities of cells, insight is expected in stabilizing normal cells and

Level of Effort: Professional Man-Years: /learning why they become malignant. approximately 3/4 man-years to date

Serial No. NCI-3264 NONE
1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Spontaneous neoplastic change during growth in culture.

Principal Investigator: R. W. Tucker

Other Investigators: K.K.Sanford, V.J.Evans, M.L.Hursey, S.L.Handleman,
T.P.Yang

Cooperating Units: None

Project Description:

Objectives: To study some of the physical and behavioral changes that cells undergo during spontaneous neoplastic change and/or long-term culture in vitro. Four objectives are the following:

1. To establish whether cells adapted to serum-free chemically defined medium are producing growth-stimulating substances required by the serum-dependent progenitor cells.
2. To assess the usefulness of the tumor-specific cytotoxicity of activated macrophages for discriminating between neoplastic and nonneoplastic cells in culture.
3. To study the differences in DNA repair capability in cell lines before and after neoplastic change.
4. To study microtubular arrays in normal and neoplastic cells.

Methods Employed:

1. Growth stimulatory substances: The media conditioned by serum independent cells is assayed for growth-stimulatory substances able to sustain growth of serum-dependent cells.
2. Cytotoxic interactions between activated mouse peritoneal macrophages and tissue culture cells will be evaluated as indicators of neoplastic change. Both endotoxin and BCG will be used for activation.
3. DNA repair will be studied by conventional autoradiographs of UV irradiated cells grown in tritiated thymidine.
4. Microtubular arrays will be measured by quantitative polarization microscopy of the birefringence of the mitotic spindle and pseudopod fibrillar material in both normal and neoplastic cells.

Major Findings:

1. Growth-stimulatory substances were not released in measurable quantity by two cell lines grown in NCTC 135.
2. Peritoneal macrophages activated by endotoxin selectively destroyed neoplastic cells in a ratio of macrophage to target cell of 10:1.
3. DNA repair in long-term cultures of mouse cells could not be adequately measured by tritiated thymidine uptake, presumably because rodents do not perform a significant amount of excision repair.
4. Investigation and selection of microscope equipment and perfusion type culture has been completed.

Significance and Proposed Course:

1. The fact that cells adapted from serum-dependent to serum-independent conditions did not release detectable growth-stimulatory substances into the medium NCTC 135 may mean that: (1) such substances were present in too low concentration to be detected, (2) the cells were producing growth-stimulatory substances not released into the medium, or (3) the cells in adaptation have changed their requirements for external growth-stimulatory substances. The first possibility is being further investigated by growing the serum-dependent and serum-independent cells in Waymouth's medium instead of NCTC 135, which provides a more sensitive assay system. Such growth-stimulatory substances might provide information concerning the serum requirements of neoplastic and nonneoplastic cells.
2. The selective toxicity of activated rat peritoneal macrophage for neoplastic cells or cells with abnormal growth patterns has to be further developed to be more specific for our tissue culture cells. Once so developed, the assay will be used to detect changes in vitro, to study mechanism of cytotoxicity in vitro, and to determine if neoplastic change or increased saturation density in vitro is the important factor underlying the selective cytotoxicity.
3. DNA repair in normal cells is not measurable by tritiated thymidine uptake because mouse cells, unlike human cells, do not use much excision repair to repair DNA damage from UV. We are investigating other approaches to this problem employing DNA isolations.
4. With the polarization microscope we can quantitate the birefringence of microtubular apparatus with change of temperature, D_2O , and colchicine, in order to define the dynamic equilibrium between oriented and non-oriented protein subunits in the mitotic spindle in both neoplastic and nonneoplastic cells. Non-mitotic spindle microtubular structures will also be studied semi-quantitatively in order to determine if the abnormal morphology, adherence, and motility of neoplastic cells (sensitive to temperature, D_2O , and colchicine) are related to the organization of microtubular apparatus.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3264

Title:

Spontaneous neoplastic change during growth in culture.

Principal Investigator: R.W. Tucker, K.K. Sanford, V.J. Evans

Section: Tissue Culture Section

Lab/Branch: Laboratory of Biology

Goal:

To characterize the physical and behavioral changes of cells during neoplastic change in culture.

Approach

- 1) Study whether cells adapted to serum-free chemically defined conditions in long-term culture produce growth-stimulatory substances required by their serum-dependent progenitor cells.
- 2) Assess the usefulness of tumor specific cytotoxicity by activated macrophages in discriminating between neoplastic and nonneoplastic cells in culture.
- 3) Study the differences in DNA repair capability in cell lines of different susceptibility to neoplastic change in vitro.
- 4) Study microtubular apparatuses in normal and neoplastic cells.

Progress:

- 1) Growth-stimulatory substances were not released in detectable amounts by cells grown in NCTC 135. Cells grown in Waymouth's medium, which provides a more sensitive assay system for such growth substances, are currently being studied.
 - 2) Preliminary experiments indicate that mouse peritoneal macrophages activated by endotoxin can selectively kill neoplastic cells in tissue culture.
 - 3) DNA repair measured by autoradiography reveals very little excision repair in mouse tissue culture cells, so that DNA isolation experiments are being investigated.
 - 4) The polarization microscope and perfusion chamber apparatus have been obtained.
- Significance for Cancer Research (NCP Objective 3 Approach 2)

Studying the spontaneous neoplastic changes occurring in tissue culture may provide new understanding of the neoplastic process itself and help therefore to develop means of controlling neoplastic change.

Level of Effort: Professional Man-Years: 1.

Serial No. NCI-3265

1. Laboratory of Biology
2. General Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Mammary Tumor Viruses in the Mouse Transmitted by Milk

Principal Investigators: G. Vlahakis and W. E. Heston

Other Investigators: G. H. Smith

Man Years:

Total:	3/4
Professional:	1/2
Other:	1/4

Project Description: Objective 1, Approach 4

Objectives: To compare three naturally occurring mammary tumor viruses in genetically similar susceptible hosts

Methods: The lines of mammary tumor viruses (MTV) that occur naturally in the different mouse strains, and that are transmitted by the milk continue to be of interest to us. Three MTV's from strains C3H, A, and DD (with their characteristic mammary tumor incidences) were all compared in genetically similar strain BALB/c hosts, which do not have MTV. The occurrence of mammary tumors in strain BALB/c breeders is approximately 20%, and the average tumor age is 16.7 months. Many of the mammary tumors are small adenoacanthomas and are usually observed at necropsy. However, when MTV is given through foster nursing on strains that have the virus, strain BALB/c is shown to be highly susceptible to the oncogenic action of MTV. For example, strain BALB/cfC3H female breeders (with C3H-MTV) have 100% mammary tumors occurring at an average age of 7.1 months. This high mammary tumor incidence is continually maintained generation after generation; the MTV is never lost. However, since breeding in general increases the occurrence of mammary tumors in mice, a group of 100 BALB/cfC3H were weaned and maintained as virgins. This would test the effectiveness of C3H-MTV in the absence of the hormones of pregnancy. We had also shown that strain BALB/cfDD female breeders (with DD-MTV) have a 97% mammary tumor incidence, occurring at an average age of 9.3 months. Like strain BALB/cfC3H, this high incidence is continually maintained. In an earlier study in which we compared strain

DD with strain C3H, we had determined that the DD-MTV was weaker either qualitatively or quantitatively than that of C3H-MTV.

To compare MTV from strain A with MTV's from strains C3H and DD, BALB/c was fostered on strain A to produce the fostered strain BALB/cfA (with A-MTV). Subsequently, groups of 54 BALB/cfA and 55 BALB/cfDD were weaned and maintained as virgins, so as to make a possible comparison with BALB/c fC3H virgins.

Almost all of the mammary tumors that occurred in both breeders and virgins throughout this study were palpable tumors and they were classified histologically according to Dunn's classification.

Results: Even when 100 strain BALB/cfC3H females (with C3H-MTV) are maintained as virgins, there were 83 (83%) with mammary tumors occurring at an average age of 11.2 months, compared with 100% at 7.1 months for the breeders. Thus, in the absence of the hormones of pregnancy, the C3H-MTV is still very effective in the production of mammary tumors in susceptible strain BALB/c females.

In the F_1 - F_7 generations of BALB/cfA (with A-MTV) there were a total of 87 breeding females. Of these there were 79 (90.8%) with mammary tumors occurring at an average age of 9.8 months. Eight without mammary tumors died at an average age of 13.4 months. Compared with BALB/cfC3H and BALB/cfDD breeders, mammary tumors in BALB/fA breeders are slightly reduced, and average tumor ages are increased but only slightly compared with BALB/cfDD.

When, however, BALB/cfA are kept as virgins, in a total of 54 there were 34 (63%) with mammary tumors occurring at an average age of 14.9 months. Compared with BALB/cfC3H virgins, this represents an appreciable reduction in mammary tumors, and an average increase in tumor age of four months. This indicates that the A-MTV is a weaker or less effective virus than C3H-MTV in the production of mammary tumors in strain BALB/c females.

In 55 virgin BALB/cfDD females, there were only 12 (21.8%) with mammary tumors occurring at an average age of 18.8 months. Forty-three died without mammary tumors at an average age of 19 months. Compared with BALB/cfC3H virgins, there was a very large reduction in mammary tumors and an approximate average increase in tumor age of 8 months. Compared with BALB/cfA virgins, there was also a large reduction in mammary tumors and an approximate average increase in tumor age of 4 months. These results clearly demonstrate that compared with both C3H and A-MTV's, the MTV of strain DD is the weakest or least effective in the production of mammary tumors in BALB/c females.

In 1971 Philomena C. Hageman and Jero Calafat of The Netherlands Cancer Institute, reported finding occasionally mammary tumor virus (MTV) antigens in mammary glands of old uninfected breeding females of strain BALB/c. When cell free homogenates or preparations from these glands were injected into young BALB/c females, they caused a high percentage of mammary tumors at six months of age. This they believe is evidence that strain BALB/c has MTV.

We now have an experiment in progress in an attempt to repeat their work with our strain BALB/c, for which we have no evidence that it has MTV. Early results seem to bear this out. Four females that had received cell free extracts of mammary glands from old uninfected BALB/c animals, were autopsied at 12 months of age without mammary tumors. We will have more information on this experiment during this year.

Substrain C57BL/He which is one of the most resistant mammary tumor strains, is genetically very resistant to the virulent strain C3H mammary tumor virus (MTV), losing it in a generation or two after its introduction. The genetically similar substrain C57BL/M of Dr. Dan H. Moore at the Institute for Medical Research, Camden, New Jersey, has now been shown to be more susceptible than C57BL/He to the development of both spontaneous and induced mammary tumors. We have now successfully developed C57BL/MfC3H (with C3H-MTV) and hope to determine how well C3H-MTV survives in this fostered strain. One C57BL/MfC3H female was necropsied at 13 months of age with a mammary tumor. Histologic examination of the tumor has shown it to be an adenocarcinoma type B. The tumor is also being examined by the electron microscope for B particles. We hope to have more information on this fostered strain later this year.

Strain C3HfBfDD (with DD-MTV) when kept as virgins, has an approximate 72% mammary tumor incidence occurring at 15 months of age. The comparable strain C3H-A^{Vy}fB (with the A^{Vy} gene) was foster nursed on strain DD to produce C3H-A^{Vy}fBfDD (with DD-MTV). Our interest in developing this fostered strain is to determine if the DD-MTV in the presence of the A^{Vy} gene will increase mammary tumors in C3H-A^{Vy}fBfDD significantly over that in C3HfBfDD, and at the same time reduce the tumor age. We are now beginning to save C3H-A^{Vy}fBfDD virgin females for this experiment.

In collaboration with Dr. Melvin Reuber of the University of Maryland Medical School, we are now planning to write a paper on the spontaneous carcinomas of the uterus that occur in our female mice. Later we plan to write another paper on the spontaneous sarcomas of the uterus in mice. Such publications should prove useful to those investigators who are working with these tumors in mice and possibly other rodents.

Significance of Program to the Institute:

The occurrence of mammary tumors in mice depend on the interplay of three factors: the genotype of the animal, hormones, and the mammary tumor virus. In this study we have compared the effects, in both breeders and virgins, of three MTV's that are found naturally in their respective hosts. Through foster nursing, the three MTV's were compared in genetically similar susceptible hosts. The C3H-MTV was clearly the most effective in the production of mammary tumors, while the DD-MTV of strain DD, which originated in Germany and Japan and probably is not related to any strain developed in the United States, is the least effective. The MTV of strain A, which is distantly related to strain C3H, is more similar to C3H-MTV than it is to DD-MTV. The results clearly demonstrate that the three MTV's in this study differ as to their oncogenic ability in relation to mammary tumors. To the extent that any of these findings may apply to man, these are the kind of results one would expect to see.

Proposed Course of Experiment:

The study on the comparison of three mammary tumor viruses is now being concluded and the results will be submitted for publication.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3265

Title: Mammary Tumor Viruses in the Mouse Transmitted by Milk

Principal Investigator: G. Vlahakis, W. E. Heston, and G. H. Smith

Section: General Biology

Lab/Branch: Laboratory of Biology

Goal: To compare three naturally occurring mammary tumor viruses in genetically similar susceptible hosts.

Approach: Three milk borne mammary tumor viruses (MTV) from mouse strains C3H, A, and DD (with their characteristic mammary tumor incidences) were all compared in genetically similar susceptible strain BALB/c females, in both breeders and virgins. Strain BALB/c which does not have MTV and has a low incidence of mammary tumors, becomes a high tumor strain when given MTV through foster nursing on strains that have it. Accordingly, fostered strain BALB/cfC3H (with C3H-MTV) was compared with fostered strain BALB/cfA (with A-MTV), and they were both compared with fostered strain BALB/cfDD (with DD-MTV) in relation to mammary tumorigenesis.

Progress: Even in the absence of the hormones of pregnancy, 100 BALB/cfC3H virgins (with C3H-MTV) had 83% mammary tumors, occurring at an average age of 11.2 months, compared with 100% at 7.1 months for the breeders. Of a total of 87 BALB/cfA breeders (with A-MTV) there were 79 (90.8%) with mammary tumors, occurring at an average age of 9.8 months. When, however, BALB/cfA are kept as virgins, in a total of 54 there were 34 (63%) with mammary tumors, occurring at an average age of 14.9 months. In 55 virgin BALB/cfDD females, there were only 12 (21.8%) with mammary tumors, occurring at an average age of 18.8 months, compared with 97% at 9.3 months for the breeders. The results clearly demonstrate that the three MTV's differ as to their oncogenic ability, in relation to mammary tumors in genetically similar BALB/c females.

Significance for Cancer Research (NCP Objective 1 Approach 4)

Mammary tumors in mice depend on the interplay of three factors: genotype of the animal, hormones, mammary tumor virus. The three naturally occurring milk borne MTV's ranged from high, to medium, to low ability in the production of mammary tumors when compared in virgin females.

Level of Effort: Professional Man-Years: 1/2

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3266

Title: Nucleic Acids and Polymerase(s) of Intracytoplasmic A particles

Principal Investigator: Gilbert H. Smith

Section: General Biology Section

Lab/Branch: Laboratory of Biology

Goal: To isolate and characterize the nucleic acids and transcriptase(s) associated with the intracytoplasmic A particle and compare them with those of the mammary tumor virus and other known oncornaviruses.

Approach: Purified intracytoplasmic A particles were disrupted in sodium dodecyl sulfate or pronase + sodium dodecyl sulfate in extraction buffer and total nucleic acid was isolated by phenolic extraction. The nucleic acid isolate was analyzed on acrylamide-agarose gels by electrophoresis. Additionally, purified nucleic acid was subjected to analytic centrifugation in linear cesium chloride gradients to compare its buoyant density with that of normal cell nucleic acid. Co-isolation experiments with tritium-labeled unrelated cellular nucleic acid were carried out to establish that cellular contamination of the purified A particle fractions was minimal or absent. Nucleic acid polymerase activity was demonstrated in purified A particle isolates.

Progress: High molecular weight DNA was found associated with purified intracytoplasmic A particles repeatedly. Efforts to show that this DNA is not cellular DNA co-isolating with the A particles have indicated that the DNA is not a result of simple contamination of the A particle fractions with nuclear fragments. The A particle DNA possessed a slightly higher buoyant density at equilibrium in cesium chloride gradients than main band nuclear DNA. Co-isolation experiments with thymidine³-H labeled DNA from mouse embryo cells have shown that less than 0.001% of the label co-isolates with intracytoplasmic A particles. A manuscript describing these observations is in progress. Studies on the polymerase activity associated with these fractions was largely done in the laboratory of Drs. Yang and Wivel and the results are still preliminary.

Significance for Cancer Research (NCP Objective 3 Approach 2)

The project is designed to further our understanding of the intracellular events occurring in mouse mammary tumor virus infection with the aim of elucidating the replicative cycle of MTV. Subsequently, this information will be utilized to clarify the tumorigenic capacity of MTV.

Level of Effort: Professional Man-Years: 1

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3267

Title: Isolation and Characterization of a DNA Replication Complex from Paired Neoplastic and Non-neoplastic Mouse Tissue Culture Cells

Principal Investigator: Dr. David G. Longfellow and Dr. R. Raymond Gantt

Section: General Biology

Lab/Branch: Laboratory of Biology

Goal: To isolate a membrane bound DNA fraction which appears to be responsible for DNA replication in vivo and to characterize the associated DNA methylase and DNA polymerase activities.

Approach: A paired normal and neoplastic mouse embryo cell line developed by Dr. Virginia Evans is used. Neoplastic conversion is induced by supplementing the culture fluid with horse serum rather than fetal calf serum.

The "replication complex" is prepared from purified nuclei by disruption in a Parr decavitation bomb and the membrane-bound DNA is isolated from the bulk DNA by differential centrifugation.

The presence of DNA methylase is detected by the ability of a cell extract to transfer a labeled methyl group from S-adenosyl methionine to an endogenous or exogenous DNA acceptor. The acid washed DNA reaction products are hydrolyzed in formic acid and the base products are analyzed by high pressure liquid chromatography.

DNA polymerase is detected by the usual methods.

A comparison of enzyme activities in the normal and neoplastic cells will be made in an attempt to determine if there are recognizable changes that can be attributed to neoplasia.

Progress: The replicating complex has been purified and a methylase has been found that is capable of methylating endogenous, homologous DNA. Other DNA sources have been tested to find a good experimental acceptor of methyl groups. The base product which is methylated has been identified as 5 methyl cytosine by high resolution, high pressure liquid chromatography.

When overall assay parameters are better understood we will begin to study the sister neoplastic cell line which is currently being developed.

Significance for Cancer Research (NCP Objective 3 Approach 3)

It is hoped that a better understanding of the effects of neoplasia on the process of DNA replication as well as a more complete understanding of the event itself will be developed. When the mechanism of normal processes of the cell are known and defective processes can be recognized, we shall be better able to begin preventive treatments.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-3268

1. Laboratory of Biology
2. Tissue Culture Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Differentiation of Enzymatic Expression in Normal and Neoplastic Tissues

Principal Investigator: Charles K. Grieshaber

Other Investigators: Harold A. Hoffman (Laboratory of Biology, NCI)
Warren H. Evans (Laboratory of Biochemistry, NCI)

Man Years:

Total: 3
Professional: 1-1/2
Other: 1-1/2

Project Description: Objective 3. Approach 3

Objectives: To study the differentiation of enzymatic activities in mouse tissues in order to understand the processes which can regulate appearance or loss of activity. Similarities and differences between those processes which regulate enzyme activities in non-differentiating cells and in normal and neoplastic growing or differentiating cells is essential to this understanding.

Methods: Mice from eight inbred strains are used as the source of tissue enzymes upon which appropriate physical, biochemical, genetic and immunochemical analyses are carried out. New Zealand white rabbits of known immunoglobulin type are used for the production of heterologous antisera.

Biochemical Genetics of RBC Catalase in Inbred Mice:

A rapid analytical method for isolating catalase from small volumes of mouse erythrocytes was developed. The technique, involving combinations of gel filtration and ion exchange chromatography, has yielded a 400-500 fold purification of the enzyme with high specific activity. Three rabbit anti-catalase antisera have been produced to date: 1) anti-RBC lysate; 2) anti-semipurified G-100 pool; 3) anti-major enzymatically active DEAE cellulose protein peak.

In the inbred strain C3H-A^{vy} carrying both normal liver and small hepatomas (2-3 mm) we have observed a 40-60% reduction in catalase enzymatic activity in the hepatoma compared to normal liver.

Proposed Course of Project:

1) Complete chemical and physical analysis (ultracentrifugal analysis, amino acid composition, kinetic analysis, etc.) of the isolated catalase protein will be carried out.

2) The isolated preparation will be used in attempts to understand cytoplasmic interaction between catalase and other molecules in an effort to determine the chemistry of observed enzymatic microheterogeneity.

3) Further studies will be undertaken to determine the rate of catalase synthesis and degradation in C3H-A^{VY} hepatomas in an effort to understand the mechanisms involved in lowered catalase activity in neoplastic tissues.

Publications:

Grieshaber, C. K. and Evans, W. H. Effect of serum on amino acid incorporation into protein of guinea pig bone marrow granulocytes. J. Reticuloendothelial. Soc. 12: 485-496, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3268

Title: Regulation of Enzyme Expression in Neoplastic Tissue

Principal Investigator: Charles K. Grieshaber and Harold A. Hoffman

Section: General Biology

Lab/Branch: Laboratory of Biology

Goal: To understand chemical and genetic interaction in expression of protein function in neoplasms

Approach: This project utilizes biochemical, quantitative genetic and immunochemical techniques for the study of the catalase enzyme system in particular. Catalase activity is known to be reduced in liver and kidney carcinomas as well as in CML leukocytes. Thus, the regulation of its activity is dependent upon the stage of cellular differentiation. The genetic control of catalase expression is under study as well as the interaction between the enzyme and cytoplasmic components which degrade the protein.

Progress: In the first nine months of the study the enzyme has been isolated, immunochemical analysis begun and direct measurement of tissue catalase content (mass of enzyme/gm) started. The enzyme activity in some tissues (RBC and kidney) is manifested in a heterogeneous form, presumably due to interaction between the enzyme and other cytoplasmic factors. In addition, a C3H-A^{VY} hepatoma has been determined to have significantly less catalase enzyme activity (40-60%) than normal C3H-A^{VY} liver tissue.

Significance for Cancer Research (NCP Objective 3 Approach 3)

This study provides a molecular model of known genetic composition for the study of enzyme and protein regulation in neoplastic tissue. Furthermore, since there are several potential points for regulation, this system may have wide significance to general processes of differentiation.

Level of Effort: Professional Man-Years: 1

SUMMARY STATEMENT
LABORATORY OF MOLECULAR BIOLOGY
DCBD, NCI
July 1, 1972 - June 30, 1973

The research effort in the Laboratory of Molecular Biology can now be divided into four general areas:

1. What is cyclic AMP's role in the conversion of a normal cell into a cancer cell?
2. How is the expression of genetic information controlled by cyclic AMP and other factors in bacterial and animal cells?
3. What is the molecular basis of mutagenesis by viruses?
4. What is the molecular basis of genetic recombination?

Cyclic AMP and Cancer--Studies with mutant viruses indicate that a change in activity of a single gene can convert a normal cell into a cancer cell. One plausible explanation for this is for the gene to control the level of a regulatory molecule that switches off or on many properties of the cell. Cyclic AMP partially fills this role. Two lines of evidence lead us to this conclusion:

1. Many transformed cells including cells recently transformed by various sarcoma viruses have low cyclic AMP levels. With temperature sensitive mutant viruses (mutant in transformation functions) the fall in cyclic AMP level precedes or occurs at the same time as morphological evidence of transformation.

2. Treatment of transformed cells with agents that raise cyclic AMP levels, restores many but not all of their properties towards normal. The cells look more normal, have increased adhesiveness, grow more slowly, are less motile and regain their ability to make mucopolysaccharides.

How are cyclic AMP levels lowered in transformation? The mechanism is complex. In general, the activity of the enzyme that makes cyclic AMP, adenylate cyclase, is decreased. In some cases the activity of the degradative enzyme, cyclic AMP phosphodiesterase, is simultaneously increased. Both these enzymes are in the plasma membrane of the cell where many of the changes in transformed cells occur. Finally, in some cases cyclic AMP is released from the cells.

Do all viruses inactivate adenylate cyclase in a similar manner? No! Wayne Anderson finds that the decrease in adenylate cyclase activity in cells infected with two closely related viruses, Schmidt-Ruppin and Bryan strain of Rous Sarcoma virus, is due to different kinetic mechanisms. We are now trying to identify the chemical nature of the changes in adenylate cyclase in these and other transformed cells.

Little is known about how cell shape, adhesiveness and motility are controlled. Contractile proteins seem likely to be involved. In studies in collaboration with R. Adelstein (NHLI) we find fibroblasts contain myosin and actin. We are now studying the interaction of cyclic AMP with these molecules from normal and transformed cells and trying to develop methods to determine precisely where the actin and myosin are located in these cells.

Control of Gene Expression--

Cyclic AMP--M. Gallo and I. Pastan have finally been able to purify large amounts of the cyclic AMP receptor (CRP) from E. coli. With this material H. Niall (Mass. General Hospital) should be able to complete the determination of the sequence of CRP. Using antibodies to CRP, W. Anderson found there are at least 4 steps between when cyclic AMP and CRP combine and when RNA polymerase is "ready" to initiate transcription of the gal operon. Cyclic AMP and CRP are also needed to initiate transcription of the lac operon, but other factors also control lac transcription. We previously showed that an unusually nucleotide, ppGpp, is a stimulator of lac RNA synthesis and M. Aboud now finds that tRNA is needed for ppGpp to act. This finding may help to explain how transcription and translation are coupled.

Gal Repressor--S. Nakanishi has been able to obtain highly purified gal repressor protein, and show in our purified gal transcription system (gal DNA, RNA polymerase, cAMP, CRP) that the repressor really does repress gal transcription. Repression is lifted by the physiological inducer of the gal operon, galactose. Nakanishi finds that the repressor acts by preventing RNA polymerase from binding to the gal promoter locus.

On some occasions RNA polymerase will begin transcribing in one operon and continue reading into other operons. In in vivo studies Adhya finds that when RNA polymerase moves into the gal operon from an upstream lambda operon, the gal repressor cannot arrest its progress. This is an interesting way to circumvent repression.

Rho--de Crombrugge and Adhya have been using the in vitro gal RNA synthesis system to study the signals that arrest RNA synthesis. They find that a protein rho, known to terminate transcription at the end of the operon, also acts inside the operon accounting for unequal synthesis of the three proteins of the operon under certain conditions. Rho also terminates transcription when a certain type of foreign DNA becomes inserted into the gal operon. Rho acts at site inside the insertion. In studies with S. Weissman (Yale) the nucleotide sequence of these rho-sensitive sites is being determined.

Chloramphenicol Acetyl Transferase (CAT)--To expand the systems available for analysis of control mechanisms, de Crombrugge, Shaw (U. of Miami), Rosner (Lab. Mol. Biol., NIAMDD) and Pastan investigated the synthesis of CAT in cell-free extracts. Using a new highly sensitive assay they found E. coli extracts would synthesize the enzyme when

programmed with DNA from the phage P1 carrying the CAT gene. Interestingly cAMP and ppGpp both increase CAT synthesis, but act independently of one another.

Genetic Recombination--For the past few years Shimada, M. Gottesman and R. Weisberg (NICHD) have been analyzing what happens when the bacterial virus λ integrates into unusual sites in the E. coli chromosome. They find that λ acts as a mutagen inactivating a variety of different genes. Such studies are important models for now viruses might cause cancer by inactivating host genes. After integrating into the host chromosome the λ phage can be rescued from the bacterial chromosome carrying with it neighboring bacterial genes. Such transducing viruses are very useful in a wide variety of biochemical studies.

Lambda--This virus mimics in many ways the behavior of transforming viruses in animal cells. Our ideas of how transforming viruses work are based on λ studies. Court and M. Gottesman are analyzing the functions involved in how λ decides to lysogenize a cell rather than kill it. Their findings suggest that proteins that regulate this process act at a single site on the λ chromosome to activate transcription of the gene product favoring lysogeny and simultaneously to inhibit synthesis of gene products favoring cell death.

Serial No. NCI-8000

1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Regulation of Gene Expression in E. Coli by Cyclic AMP

Previous Serial Number: Same

Principal Investigator: Ira H. Pastan

Other Investigators: Wayne B. Anderson, Shigetada Nakanishi, and
Mordechai Aboud

Cooperating Units: None

Man Years: July 1, 1972 through June 30, 1973

Total: 3.9

Professional: 2.9

Others: 1.0

Project Description:

Objectives: Cyclic AMP is required for the synthesis of a variety of enzymes in E. coli, most of which are inducible. Cyclic AMP acts at a transcriptional level. Our objective is to determine how cyclic AMP, cyclic AMP receptor protein (CRP) and other factors control gene activity.

Methods Employed: Studies on mRNA and enzyme synthesis have been carried out in intact cells and in cell-free extracts. Mutants which are defective in cyclic AMP metabolism have been isolated and characterized. Proteins controlling gene transcription are isolated and their mechanism of action studied.

Major Findings: We have previously determined that cyclic AMP and the cyclic AMP receptor (CRP) are required for the initiation of transcription of the lac and gal operons.

We are continuing to investigate the molecular mechanism of this reaction. To perform physical studies on CRP we have devised a modified purification method which should allow the preparation of gram quantities of CRP. Sufficient material is now available to finish determination of the amino acid sequence of CRP.

In vivo the expression of the gal operon is repressed by gal repressor. We have now prepared highly purified gal repressor. This molecule binds tightly to the gal operator site of λ gal DNA and prevents

cyclic AMP-CRP dependent gal transcription. This repression is specifically relieved by galactose and fucose, two inducers of the gal operon. The gal repressor works by competing with RNA polymerase, cyclic AMP and CRP for binding to the gal operator-promoter region. This is of particular interest because the lac repressor works by a different mechanism in the lac operon.

Another small molecule involved in regulation of lac operon expression is ppGpp. This molecule is only active in the presence of cAMP and CRP and when a crude RNA polymerase preparation is used to transcribe lac DNA. One of the factors in the crude RNA polymerase preparation required for ppGpp action is tRNA. Other factors are now being analyzed.

Significance to Biomedical Research and the Program of the Institute: In cancer cells, genes which are normally quiet become active due to some alteration in the control of gene expression. It is not yet possible to study the expression of individual genes in animal cells. Therefore our studies on the control of the transcription of lac and gal genes by cyclic AMP, cyclic AMP receptor protein, lac repressor, gal repressor and ppGpp are a basis on which to build an understanding of how gene expression is controlled in higher organisms.

Proposed Course: 1. To examine the chemical mechanism by which cyclic AMP and CRP instruct RNA polymerase to initiate gene transcription.

2. To determine the sequence of select regions of lac and gal mRNA and thereby establish if the promoter or operator regions of the gene are transcribed.

3. To search for other regulatory molecules which control gene expression and determine how they act.

Honors and Awards: None

Publications:

Pastan, I.: Cyclic AMP. Scientific American 227: 97-105, 1972.

Pastan, I.: Current directions in research on cyclic AMP. In Anfinsen, C.B., Goldberger, R.F. and Schechter, A.N. (eds.): Current Topics in Biochemistry. New York, N.Y., Academic Press, 1972, pp. 65-100.

Nakanishi, S., Adhya, S., Gottesman, M.E., and Pastan, I.: In vitro repression of the transcription of gal operon by purified gal repressor. Proc. Nat. Acad. Sci. USA 70: 334-338, 1973.

de Crombrughe, B., Shaw, W.V., Rosner, J.L., and Pastan, I.: Stimulation by cyclic AMP and ppGpp of chloramphenicol acetyl transferase synthesis. Nature-New Biology 241: 237-239, 1973.

de Crombrugghe, B., Adhya, S., Gottesman, M., and Pastan, I.:
Effect of rho on transcription of bacterial operons.
Nature-New Biology 241: 260-264, 1973.

Aboud, M. and Pastan, I.: Stimulation of lac transcription by
guanosine 5' diphosphate 2' (or 3') diphosphate and transfer RNA.
J. Biol. Chem., in press, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial No. NCI-8000

Title: Regulation of Gene Expression in E. Coli by Cyclic AMP

Principal Investigator: Ira H. Pastan

Office of the Chief

Laboratory of Molecular Biology

Goal: Cyclic AMP is required for the synthesis of a variety of enzymes in E. coli, most of which are inducible. Cyclic AMP acts at a transcriptional level. Our objective is to determine how cyclic AMP, cyclic AMP receptor protein (CRP) and other factors control gene activity.

Approach: Prepare nucleic acids and proteins required to catalyze the controlled transcription of lac and gal genes. These include cyclic AMP receptor protein, RNA polymerase, gal repressor, and tRNA. Test for presence of other proteins in extracts of E. coli that also stimulate or repress gal and lac RNA synthesis. Purify and characterize these proteins and establish how they work.

Progress: In the past year we have been able to prepare large amounts of one of these, CRP. In collaboration with Dr. Hugh Niall, Massachusetts General Hospital, Boston, we are determining its sequence. With the use of antibody to CRP we find there are at least 5 steps in cAMP action that occur before transcription is initiated by the nucleotide. We have also found that two other unusual molecules stimulate the initiation of transcription of the lac operon. These are a small nucleotide ppGpp (guanosine 2' diphosphate 5' diphosphate) and tRNA. This finding that tRNA is involved in initiation of transcription may explain how translation and transcription are coupled.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Currently we do not have any knowledge about how the expression of genetic information is controlled in animal cells. It appears that in cancer some genes are permanently activated. By understanding how expression of bacterial operons is controlled, we may develop ideas of how cancer cells are regulated.

Level of Effort: Professional Man-Years: 3.0

Serial No. NCI-8001

1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Role of Cyclic AMP in the Regulation of Growth and Morphology of Transformed and Normal Fibroblasts

Previous Serial Number: Same

Principal Investigators: Ira Pastan and George S. Johnson

Other Investigators: Wayne Anderson, Mark Willingham, Richard Carchman, Tom Russell, Robert Adelstein, and John Goggins

Cooperating Units: Laboratory of Biochemistry, National Heart and Lung Institute and Experimental Pathology Branch, National Institute of Dental Research

Man Years: July 1, 1972 through June 30, 1973

Total:	6.0
Professional:	5.0
Others:	1.0

Project Description:

Objective: To investigate how cyclic AMP regulates the growth and morphology of normal and transformed fibroblasts, and to determine the nature of the alteration of cyclic AMP metabolism in transformed fibroblasts.

Methods Employed: Cell culture and standard biochemical analyses of the enzymes involved in cyclic AMP metabolism and the synthesis of macromolecules.

Major Findings: Transformed fibroblasts grown in tissue culture have low cyclic AMP levels. They also have many characteristics that differentiate them from normal cells. These frequently include a change in morphology, a decreased adherence to substratum, an increase in growth rate, a loss of contact inhibition of growth and movement and a decreased ability to synthesize specialized macromolecules such as sulfated mucopolysaccharides. When such cells are treated with dibutyl cyclic AMP (DBC) and substances that inhibit cyclic AMP phosphodiesterases such as theophylline, many of these characteristics return towards normal.

Why are cyclic AMP levels low in transformed cells? To answer this we are characterizing the enzymes of cyclic AMP metabolism in cultured

cells. Chick embryo cells (CEF) transformed by Rous Sarcoma Virus (RSV) have decreased adenylate cyclase activity. With the Bryan High Titer strain of RSV this decrease is due to a change in the K_m ATP and an alteration in Mg^{++} activation. With the Schmidt-Ruppin strain of RSV the decrease is due to a fall in total activity. With a temperature sensitive mutant of the Bryan strain of RSV, the inactivation of adenylate cyclase occurs within 20 minutes after the cells are shifted to the "transforming" (permissive) temperature. In contrast, the inactivation of adenylate cyclase in a temperature sensitive mutant of the Schmidt Ruppin strain requires many hours.

CEF contain at least two cyclic AMP phosphodiesterases. One of these is principally located in the plasma membrane, has a high affinity for cyclic AMP and shows negatively cooperative kinetics. The second enzyme is found in the soluble fraction of the cell, probably hydrolyzes both cyclic AMP and cyclic GMP and has a low affinity for cyclic AMP. Cells transformed by the Bryan strain of RSV have increased phosphodiesterase activity which probably accounts in part for their low levels of cyclic AMP.

What controls the adhesiveness, motility and shape of cultured cells? We are investigating contractile and structural proteins in cultured cells. We find our cells contain myosin, actin and tubulin. We have developed a quantitative method for extracting myosin from these cells and are comparing the myosin from different cell lines. We are also performing ultrastructural studies on the structural components of these cells.

Host Mutants in Cyclic AMP--To distinguish the changes in transformed cells that are due to low cyclic AMP levels from those that are not, we have begun to isolate host mutants in cyclic AMP metabolism. We have isolated a number of mutants in which the level of cyclic AMP falls when they are changed to a different temperature. These mutants are now being characterized.

Significance to Biomedical Research and the Program of the Institute: Part of the reason for the abnormal behavior of transformed cells in culture is their inability to maintain normal cyclic AMP levels. If one could raise levels in these cells, one might control the growth of certain types of cancers.

Proposed Course: 1. We will examine how the transformation event alters the ability of cells to maintain normal cyclic AMP levels. We also will study how cyclic AMP works to slow growth and produce its other effects.

2. We will investigate why some cells are much less responsive to cyclic AMP than others.

Honors and Awards: None

Publications:

Otten, J., Johnson, G., and Pastan, I.: Regulation of cell growth by cyclic adenosine 3', 5'-monophosphate: Effect of cell density and agents which alter cell growth on cyclic adenosine 3', 5'-monophosphate levels in fibroblasts. J. Biol. Chem. 247: 7082-7087, 1972.

Willingham, M.C., Johnson, G.S., and Pastan, I.: Control of DNA synthesis and mitosis in 3T3 cells by cyclic AMP. Biochem. Biophys. Res. Commun. 48: 743-748, 1972.

Goggins, J.F., Johnson, G.S., and Pastan, I.: The effect of dibutyryl-cyclic AMP on synthesis of sulfated acid mucopolysaccharides by transformed fibroblasts. J. Biol. Chem. 247: 5759-5764, 1972.

Adelstein, R.S., Conti, M.A., Johnson, G.S., Pastan, I., and Pollard, T.D.: Isolation and characterization of myosin from cloned mouse fibroblasts. Proc. Nat. Acad. Sci. USA 69: 3693-3697, 1972.

D'Armiento, M., Johnson, G.S., and Pastan, I.: Cyclic AMP and growth of fibroblasts: Effect of environmental pH. Nature-New Biology 242: 78-80, 1973.

Anderson, W.B., Johnson, G.S., and Pastan, I.: Transformation of chick embryo fibroblasts by wild type and temperature sensitive Rous sarcoma virus alters adenylate cyclase. Proc. Nat. Acad. Sci. USA, in press, 1973.

Johnson, G.S., Pastan, I., Oyer, D.S., and D'Armiento, M.: Prostaglandins, cyclic AMP and transformed fibroblasts. In Raspe, G. (ed.): Advances in the Biosciences. Ellmsford, N.Y., Pergamon Press, 1973, Vol. 9, pp. 173-178.

INTRAMURAL RESEARCH SUMMARY

Serial No. NCI-8001

Title: Role of Cyclic AMP in the Regulation of Growth and Morphology of Transformed and Normal Fibroblasts

Principal Investigators: Ira Pastan and George S. Johnson

Office of the Chief

Laboratory of Molecular Biology

Goal: To determine what properties of transformed cells are due to their low level of cyclic AMP and to determine the chemical nature of the alteration in cyclic AMP metabolism in transformed cells.

Approach: We grow normal and transformed mouse, rat and chick embryo cells in culture. We transform the normal cells into "transformed" cells with viruses (Rous Sarcoma, Murine Sarcoma, SV40, Polyoma) and temperature sensitive mutant viruses. We measure cyclic AMP levels, adenylate cyclase and cyclic AMP phosphodiesterase activity before and after transformation. We study the growth rate, adhesiveness, morphology, motility and enzyme synthesis capacity of normal and transformed cells.

Progress: We find (a) the abnormal morphology, rapid growth and decreased adhesiveness of transformed cells is partly corrected by treatment of these cells with agents that raise cyclic AMP levels; (b) after transformation cyclic AMP levels are lowered; (c) after transformation adenylate cyclase activity is decreased; and (d) after at least one type of transformation, phosphodiesterase activity is increased. The latter two findings probably explain why the cyclic AMP levels are lowered in transformed cells.

Significance for Cancer Research (NCP Objective 6 Approach 3)

If we understand the nature of the defect in regulation of cancer cells, we may be able to change cancer cells back into normal cells.

Level of Effort: Professional Man-Years: 5.0

Serial No. NCI-8005

1. Laboratory of Molecular Biology
2. Section of Biochemical Genetics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: The Biochemistry of λ Int-Promoted Recombination

Previous Serial Number: Same

Principal Investigators: Susan Gottesman, Kazunori Shimada, Jurgen Schrenk
and Max E. Gottesman

Other Investigator: Robert A. Weisberg

Cooperating Unit: Laboratory of Molecular Genetics, National Institute of
Child Health and Human Development

Man Years: July 1, 1972 through June 30, 1973

Total: 3.3

Professional: 3.3

Others: 0.0

Project Description:

Objectives: Phage-induced functions promote the integration of λ DNA into the E. coli genome. Our objective is to study these functions at the genetic and biochemical level.

Methods Employed: Standard microbiological techniques and techniques developed for this project were used in this study.

Major Findings: (a) Integration of λ at unusual sites in the E. coli chromosome occurs when the normal site is deleted. This integration can result in the appearance of E. coli mutants.

(b) The functions which promote integration cannot work sequentially. Transcription is not required for the reaction.

(c) With the use of deleted E. coli strains, it is possible a) to obtain novel transducing phage and b) to analyze regions of the E. coli chromosome by deletion mapping.

Significance to Biomedical Research and the Program of the Institute: The biochemical basis of phage integration is at present completely unknown. The phage integration reaction will serve as a model system for studying the integration of oncogenic viruses in mammalian cells.

It provides information of potential value in determining the nature of oncogenic cell transformation and thus may ultimately permit the prevention of conversion of normal to tumorigenic cells. The isolation of λ transducing phage has proven useful for the in vitro analysis of bacterial transcription and control mechanisms.

Proposed Course: We have now developed a system which permits us to begin to examine the biochemical basis of λ integration. We hope to use this system to detect the activity of phage integration functions and to elucidate the nature of the reaction they catalyze. We plan to isolate new transducing phage for use in in vitro studies.

Honors and Awards: None

Publications:

Shimada, K., Weisberg, R., and Gottesman, M.E.: Prophage lambda at unusual chromosomal locations. I. Location of the secondary attachment sites and the properties of the lysogens. J. Mol. Biol. 63: 483-503, 1972.

Shimada, K., Weisberg, R., and Gottesman, M.E.: Prophage lambda at unusual chromosomal locations: II. Mutations induced by bacteriophage lambda. J. Mol. Biol., 1973, in press.

Shimada, K., Weisberg, R., and Gottesman, M.E.: E. coli mutants produced by the insertion of bacteriophage λ DNA. Genetics, 1973, in press.

INTRAMURAL RESEARCH SUMMARY

Serial No. NCI-8005

Title: The Biochemistry of λ Int-Promoted Recombination

Principal Investigators: Susan Gottesman, Kazunori Shimada, Jurgen Schrenk
and Max E. Gottesman

Section of Biochemical Genetics

Laboratory of Molecular Biology

Goal: To determine the biochemical basis of the λ integration reaction.

Approach: We have (a) isolated λ variants which undergo the integration reaction with very high efficiency and (b) developed a spheroplast system which can be used to assay the activity of the Int system on DNA from these λ variants.

Progress: We have demonstrated that (1) the Int system which catalyzed the integration of λ involves recombination between homologous E. coli and phage DNA, although the amount of homology is small; (2) the specificity of the system is not absolute; integration of λ can occur at secondary sites on the E. coli chromosome when the normal integration site is deleted; and (3) the integration reaction does not require transcription, as is popularly believed.

Significance for Cancer Research (NCP Objective 3 Approach 1)

The biochemical basis of phage integration is at present completely unknown. The phage integration reaction will serve as a model system for studying the integration of oncogenic viruses in mammalian cells. It provides information of potential value in determining the nature of oncogenic cell transformation and thus may ultimately permit the prevention of conversion of normal to tumorigenic cells. The isolation of λ transducing phage has proven useful for the in vitro analysis of bacterial transcription and control mechanisms.

Level of Effort: Professional Man-Years: 3.3

Serial No. NCI-8006

1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Control of Expression of the Galactose Operon in E. coli K12

Previous Serial Number: Same

Principal Investigator: Sankar L. Adhya

Other Investigators: Max E. Gottesman and Benoit de Crombrughe

Cooperating Units: None

Man Years: July 1, 1972 through June 30, 1973

Total:	1.3
Professional:	1.3
Others:	0.0

Project Description:

Objectives: The enzymes of the galactose operon of E. coli are needed for utilization of galactose and normal cell morphology. The control of expression of the operon is complex. It is subjected to a negative control by gal repressor and a positive control by cyclic AMP. There are indications that the galactose operon is also subject to other control systems, including control by neighboring prophage genes. Our objective is to determine the molecular basis of the entire control system.

Methods Employed: Standard microbial genetic and biochemical techniques.

Major Findings: (a) Last year we reported the isolation of three new classes of mutants of E. coli constitutive for expression of the galactose operon -- other than the previously known galactose repressor defective and operator constitutive classes. The former three classes are also gal constitutive even when the operon carries a defect in the promoter locus of the operon. Further investigations have revealed that the three types arise by mutation in the gene for cyclic AMP receptor protein (crp), the gene for cyclic AMP biosynthesis (cyaA) and the gene which controls the enzymes involved in the synthesis of capsular polysaccharides (lon). Therefore, cyclic AMP is apparently playing a dual role in the regulation of the galactose operon -- positive and negative. It is proposed, as a working hypothesis, that the negative control by cyclic AMP arises by its inhibition together with CRP of synthesis or

activity of the lon gene product. The lon gene product is an additional repressor of the galactose operon acting at a locus, different from the known cyclic AMP dependent promoter-operator complex.

(b) The gal operon is located close to the prophage λ on the chromosome of E. coli. It is known that induction of the prophage causes constitutive expression of the gal operon (escape synthesis). We have shown that one of the components of such expression is due to transcription originating in the prophage DNA and extending into gal operon. This suggests that gal repressor fails to repress when transcription is initiated from a promoter located upstream, and indicates that repressor is active only on the cognate promoter of the operator.

Significance to Biomedical Research and the Program of the Institute:

In cancer cells, the expression of some genes are permanently turned on, i.e. expressed constitutively. Our studies are aimed to understand the molecular basis of how genes are turned on and off. We are using gal as a model system. This understanding might help to prevent conversions of cells to those capable of forming cancers

Proposed Course: 1. To determine the complete structure of the gal operon.

2. To identify all the regulatory molecules involved in turning on and off of the various genes of the gal operon.

Honors and Awards: None

Publications:

de Crombrugghe, B., Adhya, S., Gottesman, M., and Pastan, I.: Effect of rho on transcription of bacterial operons. Nature-New Biology 241: 260-264, 1973.

Adhya, S., Gottesman, M., and de Crombrugghe, B.: Termination and antitermination in transcription: control of gene expression. In Proceedings of the Symposium on Control of Transcription, Bose Institute, Calcutta, India, 1973, in press.

INTRAMURAL RESEARCH SUMMARY

Serial No. NCI-8006

Title: Control of Expression of the Galactose Operon in E. coli K12

Principal Investigator: Sankar Adhya

Office of the Chief

Laboratory of Molecular Biology

Goal: To determine the complete structure and to identify all the regulatory molecules of the gal operon of E. coli.

Approach: The enzymes of the gal operon of E. coli are needed for utilization of galactose and normal cell morphology. The control of expression of the operon is complex. Our objective is to determine the molecular basis of the entire control system using standard genetic and biochemical techniques.

Progress: (a) We have found that the gal operon is subject to, in addition to a positive control by cyclic AMP and negative control by gal repressor, also negative control by cyclic AMP and the product of the gene, lon, which also controls many other enzymes involved in the synthesis of capsular polysaccharides. We propose that cyclic AMP exerts this negative control by inhibiting the activity or synthesis of the lon gene product, a putative second repressor of the gal operon. (b) The gal operon can escape from its natural repression by gal repressor, if transcription is initiated at another distant promoter located upstream from the gal operon. We are studying the molecular mechanism of this escape synthesis.

Significance for Cancer Research (NCP Objective 3 Approach 3)

In cancer cells, the expression of some genes are permanently turned on, i.e. expressed constitutively. Our studies are aimed to understand the molecular basis of how genes are turned on and off. We are using gal as a model system. This understanding might help to prevent conversion of normal cells to those capable of forming cancers.

Level of Effort: Professional Man-Years: 1.3

Serial No. NCI-8007

1. Laboratory of Molecular Biology
2. Section of Biochemical Genetics
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Positive and Negative Regulations by Bacteriophage
Lambda Gene Products

Previous Serial Number: None

Principal Investigators: Donald L. Court and Max E. Gottesman

Other Investigators: None

Cooperating Units: None

Man Years: July 1, 1972 through June 30, 1973

Total:	1.3
Professional:	1.3
Others:	0.0

Project Description:

Objectives: We would like to define the events necessary for the establishment of lysogeny in a λ -infected cell. To do this, we have studied the cII and cIII functions of λ by genetic and biochemical approaches. These two λ functions are both known to be required for efficient λ lysogeny and λ cI-repressor synthesis.

Methods Employed: Standard microbiological and biochemical techniques have been used in these studies.

Major Findings: cII and cIII mutants have been found to be deficient in the synthesis of cI repressor protein following infection and also to overproduce lytic gene proteins. cY mutants show this same phenotype but have been shown not to be mutant in a diffusible protein (like cII or cIII) but rather in a site. The action of cII and cIII function is proportional to the multiplicity of infecting cII⁺cIII⁺ phage. All of the observed phenomena can be interpreted in terms of a mechanism in which a cII/cIII protein complex acts at a site, Y, on the λ DNA to activate transcription of the cI-repressor gene from one DNA strand and inhibit transcription of lytic genes from the other DNA strand.

A protein activity has been found in extracts of λ cI⁻ infected cells that is able to bind specifically ³²P labelled λ DNA to a membrane filter. Mutations in cII and cIII remove this activity.

Significance to Biomedical Research and the Program of the Institute:
As suggested recently by Huebner and Todaro (P.N.A.S. 64: 1087, 1969) a process analogous to lysogenization may be important in carcinogenesis. The relatively simple phage λ is a perfect model to use for an in vitro study of this process. As yet little is actually known about the regulation involved at the biochemical level for even the λ lysogenization system.

Proposed Course: We now have a system wherein we can start to ask questions about the mechanical controls required for the establishment of repression in the λ system. We plan to purify this protein and determine the mechanism by which the cII and cIII products effect transcription of the repressor gene and λ lytic genes.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial No. NCI-8007

Title: Positive and Negative Regulations by Bacteriophage Lambda Gene Products

Principal Investigators: Donald L. Court and Max E. Gottesman

Section of Biochemical Genetics

Laboratory of Molecular Biology

Goal: To determine how coliphage λ controls the expression of its genome.

Approach: Lysogeny and the synthesis of λ repressor requires the expression of the λ control genes, cII and cIII. These control proteins, in addition to having a positive effect on repressor synthesis, inhibit the expression of lytic λ functions. We plan to isolate and characterize these proteins.

Progress: A protein activity which binds to ^{32}P -labelled λ DNA has been found after infection of E. coli with cII+cIII+phage. The protein is missing when cII-cIII- mutants are used, and is not identical to the repressor protein as in each case the phage were missing a functional repressor gene.

Significance for Cancer Research (NCP Objective 3 Approach 1)

A process analogous to lysogenization may be important in carcinogenesis. The relatively simple phage λ is a perfect model to use for an in vitro study of this process. As yet little is actually known about the regulation involved at the biochemical level for even the λ lysogenization system.

Level of Effort: Professional Man-Years: 1.3

Serial No. NCI-8008

1. Laboratory of Molecular Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Initiation and Termination of Transcription
of Bacterial Operons

Previous Serial Number: None

Principal Investigator: Benoit de Crombrughe

Other Investigators: Richard Musso, Sankar Adhya, Max E. Gottesman, Ira
Pastan and Judah L. Rosner

Cooperating Unit: Laboratory of Molecular Biology, National Institute of
Arthritis, Metabolism and Digestive Diseases

Man Years: July 1, 1972 through June 30, 1973

Total:	2.2
Professional:	2.2
Others	0.0

Project Description:

Objective: To understand the precise biochemical mechanisms which
control initiation and termination of transcription in E. coli.

Methods Employed: Measurement of RNA and protein synthesis in vivo
and in cell-free systems; isolation of factors active in regulation of
initiation and termination of transcription.

Major Findings: (a) Transcription of the lac and gal operons of
E. coli is known to be regulated by cyclic AMP, a pleiotropic protein, the
cyclic AMP receptor, and by operon specific repressor-inducers systems.
In addition, other factors are probably involved in controlling the
expression of these operons. Using our previously developed purified
cell-free system for synthesis of gal and lac mRNA, we have worked out
methods for the synthesis and purification of a short RNA molecule
suitable for sequence analysis. Such analysis is now being performed on
a RNA molecule about 50-75 nucleotides long, corresponding to the 5' end
of gal mRNA.

(b) Using a cell-free system we have shown that the synthesis of the
enzyme chloramphenicol acetyl transferase is regulated both by cyclic AMP
and guanosine tetraphosphate (ppGpp). ppGpp stimulates this synthesis

even in the absence of cyclic AMP, in contrast to the lac and ara systems where ppGpp is only stimulatory in the presence of cyclic AMP.

(c) Since operons can be defined as transcriptional units which can be turned on and off, one would expect transcription to terminate at the distal end of operons. A previously described factor (rho) terminates transcription in vitro by E. coli RNA polymerase when DNA of several bacteriophages is used as template. We have demonstrated (1) rho terminates transcription at the end of both the gal and lac operons in vitro; (2) in the gal operon it also promotes termination of transcription at or near the end of the first structural gene; in the lac operon transcription is terminated within the first structural gene; (3) rho terminates transcription within the DNA of some insertion mutations in the gal operon. These in vitro studies suggest that rho may produce the following events in vivo: (1) arrest transcription at the end of an operon, (2) account for the natural polarity observed in certain operons, (3) cause the severe polar properties of some insertion mutations.

Significance to Biomedical Research and the Program of the Institute:

No information is available on the nucleotide sequence of the regulatory segments of DNA. Our aim is to obtain the nucleotide sequence of the regulatory regions of the gal operon. These regions include the sites for binding of RNA polymerase, for initiation of transcription, for repressor binding, for binding and action of termination factor(s). They will also include the ribosomal binding sites on gal mRNA. Information stemming from our studies will indicate the genetic codewords for regulation of transcription. Precise understanding of gene expression in microorganisms should provide a basis for understanding how genes are controlled in normal and transformed cells.

- Proposed Course:
1. Determine the nucleotide sequences of the gal operon, both the initiation and termination regions of transcription.
 2. Search for and purify the regulatory molecules which control termination of transcription in E. coli and determine their mode of action. Study mutants affected in these functions.
 3. Search for molecules active in the coupling of translation to transcription in E. coli.

Honors and Awards: None

Publications:

Pastan, I. and de Crombrughe, B.: Control of expression of genetic information in E. coli by cyclic AMP. In Diczfalussy, E. (ed.): Karolinska Symposia on Research Methods in Reproductive Endocrinology. Stockholm, Sweden, Karolinska sjukhuset, 1972, pp. 298-306.

de Crombrughe, B., Adhya, S., Gottesman, M., and Pastan, I.: Effect

of rho on transcription of bacterial operons. Nature-New Biology 241: 260-264, 1973.

de Crombrughe, B., Shaw, W.V., Rosner, J.L., and Pastan, I.: Stimulation by cyclic AMP and ppGpp of chloramphenicol acetyl transferase synthesis. Nature-New Biology 241: 237-239, 1973.

Adhya, S., Gottesman, M., and de Crombrughe, B.: Termination and antitermination in transcription: control of gene expression. In Proceedings of the Symposium on Control of Transcription, Bose Institute, Calcutta, India, 1973, in press.

de Crombrughe, B. and Pastan, I.: Regulation of gene expression by cyclic AMP in Escherichia coli. In Davies, D.D. (ed.): Rate Control of Biological Processes. Cambridge, England, Cambridge University Press, 1973, in press.

INTRAMURAL RESEARCH SUMMARY

Serial No. NCI-8008

Title: Studies on Initiation and Termination of Transcription in Bacterial Operons

Principal Investigator: Benoît de Crombrughe

Office of the Chief

Laboratory of Molecular Biology

Goal: To understand the precise molecular events controlling initiation and termination of transcription in specific operons of E. coli.

Approach: (1) Obtain the nucleotide sequence of the regulatory segments of the gal operon. Using our previously developed purified in vitro system for transcription of the gal operon, prepare and purify a short RNA segment, corresponding to the 5' end of galRNA, suitable for nucleotide sequence analysis. Determine the N-terminal amino acid sequence of the enzyme coded for by the operator-proximal cistron of the gal operon. Devise biochemical and genetic approaches in order to transcribe the promoter-operator segment of galDNA (from an upstream initiation site). (2) Study the mechanisms of termination of transcription. Purify the factors active in termination and determine their mode of action. Isolate mutants for these factors and study the mutant proteins in vitro. Study the different types of termination signals in the DNA. Obtain the nucleotide sequence of the 3' end of gal-RNA and several bacteriophage lambda specific RNA's. Determine if these sequences are coded for by corresponding DNA sequences or added after transcription comes to a stop.

Progress: (1) The nucleotide sequence of an RNA molecule corresponding to the 5' end of galRNA, about 50-75 nucleotides long, is being sequenced. (2) The E. coli termination factor rho promotes termination of transcription at the end of the gal and lac operons, at defined sites within these operons, and within some polar insertion mutations in the gal operon. These results suggest a similar role for rho in vivo in accounting for arrest of transcription at the end of operons and for both the natural polarity observed in certain operons and the severe polar properties of insertion mutants. The existence of at least two different types of genetic termination signals has been established.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Precise understanding of regulation of gene expression in microorganisms should provide a basis for understanding how genes are controlled in normal and transformed cells.

Level of Effort: Professional Man-Years: 2.2

SUMMARY REPORT
Laboratory of Physiology

July 1, 1972 through June 30, 1973

The major research efforts of the Laboratory of Physiology include studies of the fine structure of the thyroid gland in relation to function, of the regulation of energy and water exchange in the whole animal, of the biochemical control of a number of cellular processes altered by cancer, of certain molecular and biological effects of radiation, and of immunogenetic factors involved in bone marrow transplantation after radiation.

Studies have been made of the structural changes in the thyroid epithelial cell during pinocytosis of colloid from the lumen of the follicle. Pinocytosis in its simplest form involved the formation of a flat sheet of cytoplasm which projected into the lumen of the follicle. This cytoplasmic process rolled up to form a tube which sealed itself to envelop colloid and was drawn back into the cell. The structure formed was 4 to 8 microns long on a cell with 6 micron cross section. The source of plasma membrane appeared to be microvesicles which inserted membrane at the base of the structure near where it joined the plasma membrane of the cell. The microvesicles contained peroxidase and deposited it on the plasma membrane or in the lumen at the base of the cytoplasmic process. Pinocytosis commonly but not always occurred near the lateral margin of the cell. When pinocytosis was induced the response varied remarkably from follicle to follicle. Some cells responded within 5 minutes and the time interval required to produce an intracellular droplet of colloid required 5 to 10 minutes.

Research continues on the regulation of energy exchange and water exchange, and control of food and water intake, in whole animals, particularly on the interdependence of metabolic and behavioral responses. The studies involve both normal rats and rats with the cachexia of tumor growth. Analysis of relationships between food intake and compartments of energy cost of the organism has shown that limitation of capacity for motor activity is a feasible cause for part of the hypophagia of cancer. The only known specific failure of a feeding control in cancer has been demonstrated to be failure to respond to change in nutrient density, but this is not unique to cancer. Feeding responses to hypoglycemia and to increased energy cost induced by cold appear to be normal. Part, but not all of the polydipsia that is characteristic of tumor growth has been shown to be a normal response to the tumor-induced hypophagia. The evidence being accumulated suggests that there is no essential breakdown in regulation of food and water intake during tumor growth, but that there is very active behavioral regulation of these in an attempt to compensate for catastrophic breakdown in regulation of effective energy flow through the organism.

The control of the synthesis of macromolecules is under study as one aspect of the cell's protection against virus infection. More specifically the host modification system of bacteria involving metabolic transformations

of nucleic acids and their nucleotide precursors is being investigated. In methionineless death an *E. coli* restriction enzyme catalyzes DNA degradation, apparently by recognition of unmethylated regions of DNA, which should occur in only one strand of DNA when methionine is withdrawn. However, labelled base in the median found in both strands, suggesting recombination. Other work involves study of the metabolism of pseudouridine. Previous work on pseudouridylate synthetase suggested that pseudouridine is normally converted to uracil. Further support for this comes from the failure of the reverse reaction when the enzyme is added to tRNA deficient in pseudouridylate.

The factors regulating collagen synthesis and secretion are under study in developing chick embryos and cultured fibroblasts. It was found that ascorbic acid (vitamin C) stimulates the secretion of collagen from "normal" cultured fibroblasts, but transformed lines of fibroblasts were found to have much less of a dependence on the vitamin for maximal secretion. Studies are also in progress of the correlation between the effects of dibutyryl cyclic AMP on cellular growth and morphology and its effects on collagen synthesis and secretion, in sarcoma transformed fibroblasts.

Research continues on the effects of ionizing radiation on nucleic acids, proteins and their constituents. Radioprotective and radiosensitizing agents are being studied in order to develop new approaches to increase the differential radiation sensitivity of tumors. The protective effects of certain metal ions and of their complexes in reducing the production of free radicals in gamma-irradiated DNA have been further investigated. At metal to nucleotide ratios of 1:10, a 3- to 10-fold decrease of the electron spin resonance signal due to carbon radicals was observed, independent of dose and temperature. It appears that the metal ions act by quenching excited states and/or by charge scavenging, and that either charge or energy can migrate over long distances in these solids.

The effect of metal ions on the γ -radiolysis of polycrystalline djenkolic acid was investigated in detail by electron spin resonance. This substance is of interest because it allows one to separate the effects of metal ions on the formation of carbon radicals, their thermal decay, their conversion to sulfur radicals and the γ -radiation induced destruction of the sulfur radicals. All of these processes occur in γ -irradiated proteins, where the effect of metal ions on these four different processes is difficult to disentangle. For djenkolic acid it was found that certain metal ions decrease the carbon radical formation, increase the second order rate constant for radical recombination during thermal radical decay, but in general do not affect the rate constant for the conversion of carbon to sulfur radicals. However, the radiation-induced destruction of sulfur radicals is decreased substantially by the presence of nickel ions.

Quantitative studies of recovery from sublethal irradiation in terms of ability to tolerate a second challenging irradiation show separate patterns for hematopoietic and gastrointestinal systems. Ability to tolerate other agents will also vary according to time after irradiation and system affected. An understanding of the nature and duration of effects of each treatment or combination of treatments is essential in establishing therapeutic programs which can be tolerated by the host.

Studies continue on immunogenetic factors influencing bone marrow transplantation in mice, with the goal of avoiding GVH disease, so that this procedure can be made more useful as an adjunct to heavy radiation or chemotherapy in the treatment of cancer. It was found earlier that marrow from the mother produced a less severe reaction in radiated mouse progeny than that from the father, presumably because of the persistence of tolerance for antigenic differences between mother and fetus. It was also noted that radiated daughter mice were more tolerant of mothers' marrow than radiated sons; this was true both for inbred and hybrid strains, suggesting that it is due to antigens controlled by the Y chromosome. These phenomena have now been studied by transplantation of fertilized ova of one strain into foster mothers of another. The results generally confirm the idea that phenotypic expression of the antigens of the fetus, and responsiveness as well, is reduced by material influences, but in one case involving the H-2 and Y antigens of male mice, there was no reduction, but actually an increase in responsiveness. This finding could not be confirmed by skin allograft rejection in related substrains, it deserves further study.

Serial No. NCI-905

1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Iodide-Concentrating Mechanism of the Thyroid Gland

Previous Serial Number: Same

Principal Investigator: Seymour H. Wollman

Other Investigators: Dr. Lois W. Tice, Laboratory of Experimental Pathology,
National Institute of Arthritis and Metabolic Diseases,
NIH, Bethesda, Md. (Serial No. NIAMD, LEP-10)

Man Years:

Total: 1.5

Professional: 0.5

Others: 1.0

Project Description:

Objectives: To learn about (a) the mechanism by which the thyroid gland maintains a concentration of iodide elevated above that in the blood, and (b) the mechanism by which the thyroid gland accumulates protein-bound iodide.

Major Findings: Investigation this year has been devoted to studying the distribution of peroxidase in the thyroid gland which has been stimulated to resorb colloid by macropinocytosis. The pseudopod-like extension of the apex of the cell involved in pinocytosis is always peroxidase-negative at its distal end, even when the plasma membrane of the cell is peroxidase-positive. The membrane bounding the intracellular colloid droplet formed by macropinocytosis is also peroxidase-negative. Micropinocytosis also occurs and the coated vesicles and matrix of multivesicular bodies (into which the contents of coated vesicles are emptied) are also peroxidase-positive.

Significance to Biomedical Research and the Program of the Institute:

The work reported here may be best put into Objective 3, Approach 5. It bears on questions like how is cell surface formed, how do enzymes on the cell surface get there, and to what extent are substances on the cell surface free to move on the surface, and how is cell surface removed and its possible fate.

Proposed Course: To be continued.

Publication:

Loewenstein, J. E. and Wollman, S. H.: Kinetics of isotopic equilibration of iodine in individual rat thyroid follicles. Endocrinology 92: 894-911, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-905

Title: Iodide-Concentrating Mechanism of the Thyroid Gland

Principal Investigator: Seymour H. Wollman

Section: Cancer Physiology Section

Lab/Branch: Laboratory of Physiology

Goal: To understand the mechanism for the concentration of a radioisotope of iodide. To understand the mechanism of synthesis of thyroid hormones.

Approach:

Recent approaches include autoradiography to determine sites of deposition of radioisotope in the tissue and histochemical studies to determine the location of enzymes suspected of playing a role in hormone synthesis.

Progress:

Investigation this year has been devoted to studying the distribution of peroxidase in the thyroid gland which has been stimulated to resorb colloid by macropinocytosis. The pseudopod-like extension of the apex of the cell involved in pinocytosis is always peroxidase-negative at its distal end, even when the plasma membrane of the cell is peroxidase-positive. The membrane bounding the intracellular colloid droplet formed by macropinocytosis is also peroxidase-negative. Micropinocytosis also occurs and the coated vesicles and matrix of multivesicular bodies (into which the contents of coated vesicles are emptied) are also peroxidase-positive.

Significance for Cancer Research (NCP Objective 3 Approach 5)

The work reported here may be best put into Objective 3, Approach 5. It bears on questions like how is cell surface formed, how do enzymes on the cell surface get there, and to what extent are substances on the cell surface free to move on the surface, and how is cell surface removed and its possible fate.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-906

1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Production and Properties of Transplantable Thyroid Tumors

Previous Serial Number: Same

Principal Investigator: Seymour H. Wollman

Man Years:

Total:	0.5
Professional:	0
Others:	0.5

Project Description:

Objectives: To study thyroid growth and the production and properties of transplantable thyroid tumors in the rat: changes occurring in the thyroid during growth, hyperplasia and involution; histologic types of thyroid tumors and their progression, and the correlation of histology with the biochemistry and physiology of the tumors.

Major Findings: None this year.

Significance to Biomedical Research and the Program of the Institute: Investigation of properties of cells already able to produce tumors. Investigation of relation of tumor growth to host response.

Proposed Course of Project: To be continued.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-906

Title: Production and Properties of Transplantable Thyroid Tumors

Principal Investigator: Seymour H. Wollman

Section: Cancer Physiology Section

Lab/Branch: Laboratory of Physiology

Goal: To learn about the production, properties and progression of thyroid tumors.

Approach:

Induction of tumors by feeding of a goitrogen. Changes in histology and biochemical properties followed in successive transplant generations. Identification of cell types in thyroid which are sources of the various types of tumors.

Progress:

None this year.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Investigation of properties of cells already able to produce tumors.
Investigation of relation of tumor growth to host response.

Level of Effort: Professional Man-Years: 0

Serial No. NCI-928

1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Intracellular Colloid Droplets

Previous Serial Number: Same

Principal Investigator: Seymour H. Wollman

Other Investigators: Dr. Lawrence H. Bernstein, Research Associate;
Dr. Bruce K. Wetzel, Dermatology Branch, NCI

Man Years:

Total:	1.5
Professional:	1.5
Others:	0

Project Description:

Objectives: To determine the mechanism of thyroid hormone release.

Major Findings: We have observed the morphological changes in the cell surface which occur during macropinocytosis. The process starts with the formation of a flap of cytoplasm extending from the apex of the cell into the lumen of the follicle. This rolls up to form a tube which seals at sides and ends to enclose colloid. The cytoplasmic extension then retracts into the cell. Membrane is added to the cytoplasmic extension at its base.

Significance to Biomedical Research and the Program of the Institute: This project fits most closely under Objective 3, Approach 5, having to do with cell surface and cell membrane. It also has to do with fusion of plasma membranes and cell motion.

Proposed Course of Project: To be continued.

Publication:

Wollman, S. H. and Loewenstein, J. E.: Rates of colloid droplet and apical vesicle production and membrane turnover during thyroglobulin secretion and resorption. Endocrinology 92, in press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-928

Title: Intracellular Colloid Droplets

Principal Investigator: Seymour H. Wollman

Section: Cancer Physiology Section

Lab/Branch: Laboratory of Physiology

Goal:

To determine the mechanism of thyroid hormone release.

Approach:

We have followed morphological changes occurring during the pinocytosis of thyroglobulin solutions from the lumen of the thyroid follicles using light microscopy and both scanning and transmission electron microscopy. Also used enzyme histochemistry and autoradiography to follow details of the process.

Progress:

We have observed the morphological changes in the cell surface which occur during macropinocytosis. The process starts with the formation of a flap of cytoplasm extending from the apex of the cell into the lumen of the follicle. This rolls up to form a tube which seals at sides and ends to enclose colloid. The cytoplasmic extension then retracts into the cell. Membrane is added to the cytoplasmic extension at its base.

Significance for Cancer Research (NCP Objective 3 Approach 5)

This project fits most closely under Objective 3, Approach 5, having to do with cell surface and cell membrane. It also has to do with fusion of plasma membranes and cell motion.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-919
1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Urinary Excretion Patterns in Leukemia

Previous Serial Number: Same

Principal Investigator: James C. Reid

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: This program is a developmental effort to expand the scope of urinary analysis. Its objectives are the development of a methodology to broaden greatly the chemical information which can be extracted from the urine and the evaluation of such information for potential utility from both the clinical and research standpoints. Experimentally, a search is being conducted for significant abnormalities in the excretion patterns of all detectable ultraviolet-absorbing components of a set of urine specimens collected from leukemic and control subjects. Such components include various nucleic acid congeners but are not limited thereto.

Methodology: The basic concept is that it may be feasible to construct an analytical system powerful enough to deal with the complexity of urinary composition by the application of a computational technique for multicomponent mixture analysis to urinary fractions obtained after preliminary chromatographic simplification. The data required are simply absorbance spectra, which can be obtained by a single non-specific instrumental procedure. The necessity for the application of many chemical procedures, each specific for a particular component, is eliminated thereby and the attendant reduction of labor would make it feasible to study many components simultaneously, i.e. to study patterns of excretion. Moreover, techniques which have been developed in this program permit quantitative estimation of the components without the necessity for chemical identification, a feature which facilitates further the recognition of pattern abnormalities if they should occur.

The preliminary chromatographic simplification is effected by a system of ion-exchange column procedures developed earlier in the course of this program. The system reduces each urine specimen to approximately fifty fractions. Each fraction is a mixture whose components typically number about a half-dozen, and in general the individual components can be obtained in small amounts by paper chromatography. The absorbance spectra of the isolated components then serve as the basis for computing the composition of the corresponding fraction obtained from other urinary specimens.

Status: A heuristic procedure, described earlier, for selecting the best set of data points for a multicomponent analysis has been subjected to some further exploration. A change in computer facilities has interrupted the work and an adequate evaluation of the procedure is not available.

Significance to Biomedical Research and the Program of the Institute:
National Cancer Plan objective 5 (diagnosis), approach 4.

Proposed Course: Reprogramming of the software necessary for the analysis of the data on hand would be a major undertaking and the future course of this project is uncertain. Alternative approaches to the analytical problem are being considered.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-919

Title: Urinary Excretion Patterns in Leukemia

Principal Investigator: James C. Reid

Section: Cancer Physiology Section

Lab/Branch: Laboratory of Physiology

Goal:

To expand the scope of clinical urinary analysis.

Approach

Liquid column chromatography complemented by computer-based multicomponent analysis of mixed chromatographic fractions.

Progress:

Computational feasibility has not been established.

Significance for Cancer Research (NCP Objective 5 Approach 4)

A urinary test which would reveal the presence of cancer would have obvious utility for population screening and for improving prognosis through earlier detection.

Level of Effort: Professional Man-Years: 1

1. Laboratory of Physiology
2. Cancer Physiology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Protein Synthesis and Its Control in Normal and Tumor Cells

Previous Serial Number: Same

Principal Investigator: Marco Rabinovitz

Other Investigators: Dr. Satoshi Mizuno, Visiting Fellow to March 15, 1973
Dr. Saburo Ayukawa, Visiting Fellow from March 15, 1973
Dr. Kenneth Balkow, Visiting Fellow

Man Years:

Total:	6.5
Professional:	3.0
Others:	3.5

Project Description:

Objectives:

a) To study, in both normal and tumor cells the controls exercised by these cells in regulating the synthesis of protein. This is generally made through an analysis of the biochemical lesions introduced within the pathway by deficiencies of essential nutrients or by inhibitors.

b) Where applicable, to extend the information obtained in the first objective to the tumor-bearing animal under controlled nutritional conditions, with the view of specific inhibition of protein synthesis by cancer cells or to other selective lethal effects.

Major Findings:

Previous studies in this laboratory have demonstrated that a translational repressor which inhibits peptide chain initiation, is formed in the cytoplasm of hemin deficient reticulocytes and that this markedly reduces the rate of globin synthesis. The inhibition of globin synthesis in cell-free reticulocyte preparations by this hemin-controlled repressor can be overcome by addition of an initiation factor isolated from reticulocyte ribosomes. Our principal efforts this year have been directed toward further localizing the site of action of the repressor and understanding the biochemical basis of the relief of inhibition by initiation factor. Towards this end, several assay systems,

involving the partial reactions of peptide chain initiation, have been developed and the action of the repressor on these partial systems evaluated. Furthermore the stability and distribution of the initiation factor on ribosomal subunits obtained from normal and inhibited systems has been determined.

The following characteristics of the initiation factor are not affected by the translational repressor:

1. Activity in promoting protein synthesis by systems containing potassium chloride extracted ribosomes, which lack endogenous initiation factor.
2. Activity as a dissociation factor for reticulocyte ribosomes in systems active in protein synthesis.
3. Activity in the formation of a ternary complex between the initiation factor, met-tRNA_f and GTP.
4. Activity in promoting met-tRNA_f binding to the small ribosomal subunit in hemin deficient lysate systems.
5. The distribution of the factor between the small and large native ribosomal subunits in lysates under normal, hemin deficient or inhibited conditions.

A partial reaction of peptide chain initiation which is sensitive to both hemin deficiency and the hemin controlled repressor is the interaction between the native ribosomal subunits and the trinucleotide codon, AUG, to give monoribosomes. The inhibition of this reaction by the repressor is not blocked by initiation factor. A sensitive assay for repressor activity has been developed with the use of a system involving dissociation of free ribosomes into subunits with initiation factor, followed by codon directed reassociation to monoribosomes.

The overall results indicate that the hemin-controlled repressor does not have a direct action on the initiation factor, but that each, respectively inhibit and promote peptide chain initiation by independent routes.

Significance to Biomedical Research and the Program of the Institute: These studies are designed to help explain the nature of the lesions caused by nutritional deficiencies and antagonisms at the level of macromolecular biosynthesis. Such evaluations should provide a basis for our understanding of control of protein-forming systems in various cell types. This project conforms to the NCI research plan as follows: Objective 3, Approach 1, nature and modification of precancerous state to determine mechanisms accounting for high degrees of stability of cell functioning.

Publications:

Mizuno, S., Fisher, J. M., and Rabinovitz, M.: Hemin control of globin synthesis: Action of an inhibitor formed in the absence of hemin on the reticulocyte cell-free system and its reversal by a ribosomal factor. Biochim. Biophys. Acta 272: 638-650, 1972.

Gross, M., and Rabinovitz, M.: Control of globin synthesis by hemin: Factors influencing formation of an inhibitor of globin chain initiation in reticulocyte lysates. Biochim. Biophys. Acta 287: 340-352, 1972.

Gross, M., and Rabinovitz, M.: Control of globin synthesis in cell-free preparations of reticulocytes by formation of a translational repressor that is inactivated by hemin. Proc. Natl. Acad. Sci. USA 69: 1565-1568, 1972.

Gross, M., and Rabinovitz, M.: Partial purification of a translational repressor mediating hemin control of globin synthesis and implication of results on the site of inhibition. Biochem. Biophys. Res. Commun. 50: 832-838, 1973.

Mizuno, S., and Rabinovitz, M.: Factor-promoted dissociation of free ribosomes in a rabbit reticulocyte lysate system: Inhibition and requirement for an energy source. Proc. Natl. Acad. Sci. USA 70: 787-791, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-925

Title: Protein Synthesis and Its Control in Normal and Tumor Cells

Principal Investigator: Marco Rabinovitz

Section: Cancer Physiology Section

Lab/Branch: Laboratory of Physiology

Goal: To understand the mechanisms by which normal and tumor cells regulate the synthesis of protein.

Approach: The requirement for iron or hemin for the synthesis of globin, the protein moiety of hemoglobin, may be considered from both a nutritional and regulatory point of view in its effect on macromolecular synthesis. This role of hemin for globin synthesis is readily demonstrable both in intact rabbit reticulocytes and their cell-free preparations. Since the control mechanism is preserved under suitable acellular conditions, biochemical tracer and separation techniques can be utilized in attempts to understand the molecular mechanisms involved in this control.

Progress: During hemin deficiency, globin synthesis in rabbit reticulocytes is retarded by translational repressor(s) which inhibit peptide chain initiation. These are proteins and are formed in the ribosome-free supernatant fraction from available precursor, pro-repressor. Hemin blocks this conversion. The repressor inhibits peptide chain initiation by interfering with the attachment of met-tRNA_f to the small ribosomal subunit. This is consistent with the observation that synthesis of minority proteins is also inhibited. The inhibition is reversed by an initiation factor obtained from reticulocyte ribosomes. Repressor neither inactivates nor influences the availability of the initiation factor, but inhibits initiation codon dependent interaction of the two ribosomal subunits.

Significance for Cancer Research (NCP Objective 3 Approach 1)

These studies help explain the nature of the lesions caused by nutritional deficiencies and antagonisms at the level of macromolecular biosynthesis. Such evaluations provide a basis for our understanding of control of protein-forming systems in various cell types. This project conforms to the NCI research plan as follows: Objective 3, Approach 1, to determine the nature of mechanisms accounting for high degrees of stability of cell functioning.

Level of Effort: Professional Man-Years: 3

1. Laboratory of Physiology
2. Energy Metabolism Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Total Metabolism

Previous Serial Number: Same

Principal Investigator: Seoras D. Morrison

Man Years:

Total:	3
Professional:	1
Others:	2

Project Description:

Objectives:

(a) Investigation of the patterns of heat production of rats with concomitant recording of feeding behavior and other activity with a view to finding how the energy expenditure, feeding and general behavior pattern are related to tumor growth and to finding the nature of the loads imposed by a tumor on the host.

(b) Investigation of relative changes in water and material exchanges in tissue compartments of the rat during imposed and induced changes in food and water intake and the relation of these to tumor induction and growth.

(c) Examination of possible experimental methods of simulating in non-tumor-bearing rats the cachexia and decline of food intake found during neoplastic growth.

(d) Development of quantitative and reproducible assessment of behavioral output, to yield more sensitive and earlier detection of systemic effects of tumors.

Methods Employed: The methods of indirect calorimetry, operant conditional responses, placement of electrolytic lesions in or stimulation of the central nervous system, continuous or programmed infusions into unrestrained animals, and methods of orthodox nutritional studies. Ballistic methods of recording and partitioning total motor activity. Computer methods of numerical analysis of serial records of tumor growth and of continuous

records of gaseous exchange. Sprague-Dawley rats and Buffalo rats are used in all animal experiments. Most tumor-bearing rats are inoculated with Walker 256 carcinoma, but other transplantable tumors are also used.

Major Findings:

(a & b) Work on objective (a) and (b) above, has been effectively halted during the past year by the necessity of moving quarters and having these suitably altered, and by delays in obtaining suitable replacement equipment. Most work has been concentrated on the following:

(c) Work on feeding response to food dilution during tumor growth has been extended to long-term imposition of a diluted diet regimen. A growing tumor depresses the acute feeding response to nutrient dilution (Cancer Res. 32: 968-972, 1972). The depression is less severe in chronic diet dilution and is eventually partially offset by a secondary control response not so far identified (Cancer Res. 33: 526-528, 1973). Response to hypoglycemia or glucoprivia does not appear to be affected by tumor growth. This is the first time a specific feeding regulatory failure has been identified in tumor growth.

Work has been continued on differential effects of host strain on various feeding and drinking responses and metabolic characteristics (Am. J. Physiol., in press, 1973), with a view to analyzing differential effects of host strain and tumor type in development of cachexia. This work has now been extended to study patterns of energy flow in different tumor-bearing organisms and the feeding and drinking responses to change in environmental temperature.

(d) A method allowing discrimination between feeding activity, feeding efficiency and food intake has been developed and applied to the analysis of some normal controls of food intake (J. Appl. Physiol. 34: 268-270, 1973). This method is now being applied to analyze the nature of feeding change in growth of three types of tumor and the interaction of these with other induced changes in food intake. Preliminary results indicate that extensive reorganization of feeding behavior in response to tumor is able to delay the appearance of overt cachexia until long after the cachectic process has begun.

Significance to Biomedical Research and the Program of the Institute:

The findings on partition of energy expenditure and of tissue compartments and on processes influencing food and water intake make it possible to examine some features of tumor-host relationships that have not previously been subject to analysis. The methods and findings on sensitive and early changes in systemic response to tumors should be utilizable in refined animal testing of therapeutic agents. It is believed that the work is throwing useful light on the reasons for the rapid and extreme debilitation of the host during tumor growth. The section of the National Cancer Plan that the work most closely approximates is: Rehabilitation, Objective 7 (To restore patients with residual deficit as a consequence of their disease

or treatment to as nearly a normal functioning state as possible). Approach 1 (Develop improved means to provide treatment which will encompass physiological aspects of cancer).

Proposed Course of Project: Work on energy and water exchange of tumor bearers will be continued along with study on the interaction of metabolic and behavioral responses of normal and cancerous animals, with particular reference to the reasons for the changes in food and water intake and depletion of host tissues that occur during tumor growth.

Publications:

Morrison, S. D., and Coffey, N. F.: Feeding activity and feeding efficiency as distinct modes of change in food intake. J. Appl. Physiol. 34: 268-270, 1973.

Morrison, S. D.: Control of food intake during growth of a Walker 256 carcinosarcoma. Cancer Res. 33: 526-528, 1973.

Morrison, S. D.: Differences between rat strains in metabolic activity and in control systems. Am. J. Physiol., 1973. In press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-944(a)
Title: Total Metabolism

Principal Investigator: Seoras D. Morrison
Section: Energy Metabolism Section
Lab/Branch: Laboratory of Physiology
Goal: Analysis of the determinants of control of energy and water exchange in the whole animal, and the application of this to the genesis and course of cancer cachexia.
Approach:

The specific topics of investigation are the control of energy and water exchange and of food and water intake. The methodologies utilized include whole animal respirometry and calorimetry, central nervous (diencephalic) interference, function ablation, behavioral methods and selective behavioral deletion, and orthodox nutritional methods.

Important characteristics of the pattern and distribution of energy flow in normal animals have been established and changes in these in the tumor-bearing organism have been quantitated. Regulatory failure in food and water intake and changes in feeding behavior induced by a tumor are being examined. The temporal and causal relations between changes in energy flow and change in behavior and control are being investigated.

Progress:

For the first time, specific regulatory deficits (failure to respond to nutrient density change) and behavioral modification (change in motor organization of feeding) in response to tumor growth have been identified. These changes occur well before the appearance of overt cachexia and before most overt signs of the cancerous process.

Significance for Cancer Research (NCP Objective 7 Approach 2)

The project identifies and analyzes the regulatory abnormalities of the cancerous host with a view to modifying these.

Level of Effort: Professional Man-Years: 1

1. Laboratory of Physiology
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mathematical Programming

Previous Serial Number: Same

Principal Investigator: Florence K. Millar

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: To facilitate the investigation of biomedical research problems through the application of computer programming and computer data processing. Many of the projects reported here are collaborative efforts with investigators in other units of NCI and NIH.

Methods Employed: The IBM-1620 facility of the NCI has been described in previous reports [see NCI-944(b), 1969]. For some computer applications, use has been made of the central NIH computer facility (Computation and Data Processing Branch, DCRT).

Major Findings:

Computer Operations:

The 1620 has been operated as before. Computer operations include routine procurement of supplies, scheduling of user time and maintenance, and making additions to the system software as needed.

Computer Applications:

- (1) Processing of data from liquid scintillation spectrometers.

The 1620 has been used to reduce and process the raw data from single- and double-label experiments as follows:

Buoyant density determinations of DNA from E. coli
(Dr. T. R. Breitman, Dr. B. Bindslev)

Analysis of arbovirus proteins and RNA species
(Dr. J. G. Levin, C/LP)

(2) Statistical analysis and modelling

Standard statistical methods, including simple linear and multiple regression analysis, have been applied. In studies of enzyme kinetics, use has been made of programs developed by Cleland (Nature 198: 463, 1963) and of other curve-fitting techniques available at the DCRT to handle the non-linearity of the reaction equations.

Studies for which the computer has application in the general area of statistics and modelling are as follows:

Effect of ionizing radiation on DNA, protein, and their constituents
(Dr. P. Riesz, Dr. E. Rotlevi, Mr. H. M. Moss, C/LPHY)

Simulation of ESR spectra from parameters (Program obtained from external source) (Dr. E. Rotlevi, C/LPHY)

Kinetic studies on UMP-CMP kinase and on uridine-cytidine kinase
(Dr. E. P. Anderson, Dr. T. Q. Garvey, Mr. A. S. Liacoura, C/LB)

Kinetic studies on pseudouridylate synthetase from E. coli
(Dr. T. R. Breitman, Dr. D. R. Krauss)

Simulation of density gradient curve
(Dr. M. Rabinovitz, Ms. J. M. Fisher, C/LPHY)

Measurement of cyclic AMP levels in tissues
(Dr. A. Peterkofsky, Ms. C. Gazdar, H/LBG)

Calculation of fiber particle data
(Dr. M. F. Stanton, Ms. C. P. Wrench, C/LP)

(3) Processing of data from energy metabolism studies

Further programming has been done for the analysis of paper tape output from

the data acquisition system to be used with the small animal calorimeter (Dr. S. D. Morrison, C/LPHY)

(4) Preparation for transfer of operations to PDP/11 computer

Utility programs have been written to adapt existing software for use on the newly acquired PDP/11 computer of the Mathematical Biology Section, Laboratory of Theoretical Biology, NCI.

Significance to Biomedical Research and the Program of the Institute: Mathematical and computer programming applications contribute to the investigation of research problems by providing (1) accurate and efficient methods for handling laborious calculations of data and visualizing results, (2) the ability to store and retrieve large amounts of data, and (3) opportunities for testing mathematical models based on experimental results. This project most closely supports Objective 4, Approach 4 of the "National Cancer Plan" by providing methods of developing mathematical models of the cancerous process.

Proposed Course of Study: The IBM-1620 computer system will have been phased out by the end of FY73 and this project will be completed at that time. Computer applications will continue as part of the program of the Mathematical Biology Section, Laboratory of Theoretical Biology, NCI.

Publication:

Garvey, T. Q. III, Millar, F. K., and Anderson, E. P.: Adenosine triphosphate: uridine monophosphate-cytidine monophosphate phosphotransferase from *Tetrahymena pyriformis*. II. Kinetic studies and reaction mechanism with UMP. Biochim. Biophys. Acta 302: 30-49, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-944(b)

Title: Mathematical Programming

Principal Investigator: Florence K. Millar

Section: Office of the Chief

Lab/Branch: Laboratory of Physiology

Goal: To provide computational and mathematical assistance for the analysis of bio-medical research data.

Approach:

Applications programming and use of computer facilities of NCI and DCRT

Progress:

Applications have included studies on biosynthetic mechanisms, mathematical modelling of enzyme kinetics, statistical analysis of data, and preparation for analysis of data obtained in whole animal calorimetry.

Significance for Cancer Research (NCP Objective 4 Approach 4)

This project provides procedures for developing mathematical models of mechanisms related to the cancerous process.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-945

1. Laboratory of Physiology
2. Physical Biology Section
3. Bethesda, Md.

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Study of the Factors Regulating the Synthesis of Collagen during Embryonic Development and Growth of Cultured Fibroblasts

Previous Serial Number: Same

Principal Investigator: Beverly Peterkofsky

Other Investigators: Robert Diegelmann, PHS Fellow, Guest Worker
Thomas Blanck, Research Associate

Man Years:

Total:	4.5
Professional:	2.0
Others:	2.5

Project Description:

Objectives: To elucidate the basic mechanisms of collagen synthesis and secretion and to investigate the factors which regulate these processes in cultured fibroblasts, both normal and transformed, and during embryonic development.

Methods Employed: Proteins of whole chick embryos, isolated bones of these embryos, cell-free systems derived from these bones or cultured fibroblasts are labeled with ^{14}C -proline. The proteins are precipitated with trichloroacetic acid, redissolved and then assayed for radioactivity in collagen and noncollagenous proteins by a method involving specific digestion of collagen in a mixture of proteins using highly purified collagenase. This method was developed in our laboratory and is described in a previous report (1968-9). The relative rate of collagen synthesis can be calculated from data obtained by this method and the specific rate of synthesis in tissues or cells is calculated by determining the DNA content and expressing the rate as cpm/ μg DNA. The proteins of the cell and medium fractions of cultured fibroblasts are analyzed separately in order to study secretion. Morphological changes in cultured cells are recorded by polaroid photomicroscopy.

Cell-free incorporation of ^{14}C -proline is studied with a system consisting of membrane-bound or free polysomes derived from chick embryo

bone tissue and soluble factors derived from chick embryo liver, a tissue which synthesizes very little collagen, so that variations in collagen-synthesizing polysomes of a tissue may be studied independently of possible variations in the soluble fraction.

Major Findings:

I. Collagen synthesis in a cell-free system

A cell-free system consisting of polysomes from embryonic bone tissue and soluble fraction from embryonic liver was found to synthesize collagen. In several connective tissues such as tibia, femur, frontal bone, skin and cultured chick embryo bone fibroblasts, 70-90% of the collagen synthesizing activity was present in polysomes bound to the membranes of the endoplasmic reticulum. This fraction was characterized by electron microscopy and by showing that detergent treatment dissociated the collagen synthesizing activity from the membranes. It was also shown that in the absence of the cofactors required for the hydroxylation of proline and lysine in collagen (ascorbic acid, Fe^{++} and α -ketoglutarate), unhydroxylated collagen was synthesized by polysomes whether they were attached to membranes or dissociated by detergent treatment. When hydroxylase cofactors were added to prelabeled, unhydroxylated collagen attached to membrane-bound polysomes hydroxylation occurred, however, no hydroxylation of proline could be obtained in collagen prelabeled on detergent dissociated polysomes, suggesting that prolylhydroxylase is associated with the membrane fraction and may play a role in regulating collagen secretion, the first step of which is probably transfer into the cisternae of the endoplasmic reticulum.

II. Effect of ascorbic acid on collagen synthesis and secretion in cultured fibroblasts

We had previously found that in L-929 cells, a line of transformed mouse embryo fibroblasts, ascorbic acid was required for hydroxylation of proline in collagen, but the vitamin did not affect the rate of synthesis of collagen nor its secretion. Since it has long been known that in scurvy, collagen synthesis as judged by histological and electron microscopic examination, is greatly reduced, we concluded that L-929 cells lack some regulatory mechanism present in normal connective tissue cells. Therefore, we examined collagen synthesis and secretion in cells more closely related to "normal" cells: Balb 3T3 fibroblasts, a contact inhibited line of embryonic mouse cells, and primary cultures of chick embryo fibroblasts derived from frontal bone. The major findings may be summarized as follows:

- a. Collagen secretion into the culture medium is stimulated by the presence of ascorbic acid in both 3T3 and chick embryo fibroblasts.
- b. In 3T3 cells, the absence of ascorbic acid also results in marked underhydroxylation of proline in collagen, suggesting, but not proving conclusively, that it is this underhydroxylation which leads to reduced secretion. This is a tempting conclusion to

draw, since it has been found by others that inhibition of proline and lysine hydroxylation by an iron chelator leads to inhibition of collagen secretion.

- c. Although the rate of collagen synthesis was not affected by ascorbate deprivation in 3T3 cells, there was a two-fold reduction in the relative rate of collagen synthesis in bone fibroblasts incubated in the absence of ascorbate. These data suggest that a decreased rate of secretion leading to intracellular accumulation of collagen may lead to a feed-back inhibition of collagen synthesis and this would explain the decreased rate of synthesis of collagen observed in scurvy.

We have since found that ascorbate also stimulates collagen secretion in human diploid fibroblasts but exerts a much less marked stimulation in Kirsten sarcoma transformed cells, Ki-3T3, which further suggests that transformed cells such as L-929 and Ki-3T3 have lost a regulatory mechanism.

III. Effect of dibutyryl cyclic AMP on the growth, morphology and rate of collagen synthesis of Kirsten sarcoma virus transformed 3T3 fibroblasts (Ki-3T3)

It was found that, as in DNA virus transformed fibroblasts, dibutyryl cyclic AMP inhibited the growth and altered the morphology of the RNA virus transformed Ki-3T3. These effects were proportional to the concentration of dibutyryl cyclic AMP. It was also found that after 2-3 days of growth in the presence of the chemical, both collagen and noncollagenous protein synthesis increased but collagen synthesis increased preferentially so that its relative rate of synthesis was about three times higher than in control cells.

Significance to Biomedical Research and the Program of the Institute:

Determining the precise mechanism by which vitamin C affects collagen synthesis should lead to a more accurate use of this vitamin in general and especially in cases where surgery has been used extensively, as in cancer patients. Our studies with dibutyryl cyclic AMP are aimed at determining the extent to which this chemical reverses cell transformation.

Proposed Course of Research:

- I. Mechanism of collagen synthesis utilizing a cell-free system consisting of membrane-bound polysomes

This system affords an opportunity to investigate the initial steps in collagen synthesis and secretion and will be studied in greater detail.

- II. Developmental studies

The cell-free system will also be used to determine the mechanism by which collagen synthesis is increased in bone tissue during development.

Polysomes will be isolated from bone tissue of various aged embryos and tested for activity.

III. Effect of ascorbic acid on collagen secretion

The correlation between the effect of ascorbate on proline and lysine hydroxylation and the effect of the vitamin on secretion will be further investigated to determine whether this is the mechanism by which ascorbate regulates secretion.

IV. The effect of dibutyryl cyclic AMP on collagen synthesis and secretion in Ki-3T3 cells

The stimulatory effect of dibutyryl cyclic AMP on the incorporation of ^{14}C -proline into collagen will be investigated in greater detail to ascertain whether the effect is attributable to increased synthesis, decreased degradation or perhaps an increase in the transport into the cell of labeled proline. Further correlations between effects of the nucleotide on growth, altered morphology and collagen synthesis will be sought.

Publications:

Peterkofsky, B.: The effect of ascorbic acid on collagen polypeptide synthesis and proline hydroxylation during the growth of cultured fibroblasts. Arch. Biochem. Biophys. 152: 318-328, 1972.

Peterkofsky, B.: Regulation of collagen secretion by ascorbic acid in 3T3 and chick embryo fibroblasts. Biochem. Biophys. Res. Commun. 49: 1343-1350, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-945

Title: Study of the Factors Regulating the Synthesis of Collagen during Embryonic Development and Growth of Cultured Fibroblasts

Principal Investigator: Beverly Peterkofsky

Section: Physical Biology Section

Lab/Branch: Laboratory of Physiology

Goal: To elucidate the basic mechanism of collagen synthesis and secretion and the factors which regulate these processes in cultured fibroblasts, both normal and transformed, and in embryonic development.

Approach:

Collagen synthesis is measured by labeling total protein of embryonic tissues or cells in culture with ^{14}C -proline and then specifically digesting the collagen with a highly purified preparation of the enzyme collagenase. Cell-free synthesis is studied similarly by using polysome preparations derived from embryonic bone tissue to synthesize collagen. Secretion is studied in cell cultures by analyzing the proteins of the medium and cell fractions separately. The effects of various chemicals such as ascorbic acid or cyclic AMP derivatives on collagen synthesis specifically and on general protein synthesis as well as on collagen secretion can then be analyzed.

Progress: We have found that ascorbic acid (vitamin C) stimulates the secretion of collagen from the contact-inhibited cell line Balb 3T3 as well as from primary cultures of chick embryo and human diploid fibroblasts. In contrast, the chemically transformed line L-929 and Kirsten sarcoma virus transformed 3T3 cells (Ki-3T3) showed almost no dependence on ascorbate for maximal secretion. The chemical dibutyryl cAMP which inhibits the growth and alters the morphology of Ki-3T3 also preferentially stimulates collagen synthesis and reimposes dependence on ascorbate for maximal secretion, bringing it closer to the behavior of the parent 3T3 cells. In addition, it was found, using a cell-free system, that collagen synthesis occurs mainly on polysomes bound to the membranes of the endoplasmic reticulum and that the enzyme which forms hydroxyproline from proline in collagen is probably also attached to these membranes.

Significance for Cancer Research (NCP Objective 3 Approach 1,3)

Determining the precise mechanism by which vitamin C affects collagen synthesis should lead to a more accurate use of this vitamin in general and especially in cases where surgery has been used extensively, as in cancer patients. Our studies with dibutyryl cyclic AMP are aimed at determining the extent to which this chemical reverses cell transformation.

Level of Effort: Professional Man-Years: 2

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-948

Title: Control of Macromolecular Synthesis

Principal Investigator: Theodore R. Breitman

Section: Physical Biology Section

Lab/Branch: Laboratory of Physiology

Goal: To understand better the mechanisms that mammalian cells have developed to prevent virus infection.

Approach:

The assumption has been made that the host modification system described in bacteria will have its counterpart in mammalian cells. In order to become more familiar with the techniques involved in such studies we are studying the host modification system of E. coli 15. Our approach has been to understand the action of the restriction enzyme from both experiments in vivo (with intact cells) and in vitro (by isolation and characterization of the enzyme).

Progress:

The techniques involved in this study have been perfected. These techniques include CsCl density gradient centrifugation, isolation of circular DNA, and the accumulation of mutants defective in host modification.

Significance for Cancer Research (NCP Objective 3 Approach 1)

The host modification system of bacteria functions to protect the cell from virus attack. I assume that a similar mechanism will also be found in mammalian cells.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-948

1. Laboratory of Physiology
2. Physical Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Control of Macromolecular Synthesis

Previous Serial Number: Same

Principal Investigator: Theodore R. Breitman

Other Investigators: David R. Krauss, Research Associate
Birgitte Bindslev, Visiting Fellow

Man Years:

Total: 4
Professional: 2
Others: 2

Project Description:

Objectives: To clarify metabolic transformations of nucleic acids and their nucleotide precursors.

Major Findings:

A. The metabolism of pseudouridine

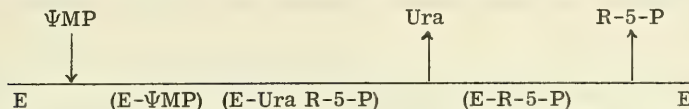
1. The purification and kinetic analysis of pseudouridylate synthetase was completed. This enzyme catalyzes the reversible reaction:



The equilibrium constant $\left[\frac{(\text{uracil}) \times (\text{ribose-5-P})}{\Psi\text{MP}} \right]$ is 3×10^{-4} M. Thus, the reaction is in the direction of pseudouridylate degradation, a finding consistent with the metabolic role of this enzyme discussed in previous Annual Reports.

The K_m values for the three substrates were: ribose-5-P, 24 μM ; uracil, 540 μM ; and ΨMP , 9.1 μM .

Kinetic analysis of the enzyme indicates that its mechanism is a sequential Ordered Uni Bi and the following model for its action can be constructed:



2. Attempts were made to determine if pseudouridylate synthetase was involved in the formation of Ψ MP at the tRNA level. For these experiments, tRNA which is deficient in Ψ MP was isolated from a mutant of Salmonella typhimurium. Addition of pseudouridylate synthetase was found to have no effect on the conversion of uridylate residues to pseudouridylate residues in this tRNA.

3. Studies were conducted on 4-thiopseudouridine (4-thio Ψ rd), an analog of pseudouridine. This analog was synthesized by and obtained from Dr. Paul Wigler of the University of Tennessee. It was found that 4-thio Ψ rd was a potent inhibitor of growth of those mutants of *E. coli* that could grow on pseudouridine as a pyrimidine source. From many different growth experiments it was concluded that 4-thio Ψ rd interferes with both the uptake as well as the enzymatic conversion of pseudouridine to uracil.

B. Methionineless death in *E. coli*

In previous Annual Reports (1970-1972) the phenomenon and biochemical basis for methionineless death was described. It was found that the DNA degradation associated with this phenomenon was catalyzed by the action of the E. coli 15 restriction enzyme which apparently recognizes unmethylated regions of DNA. When a culture of E. coli is incubated in the absence of methionine, rounds of DNA replication in progress are completed but no new rounds of replication are initiated. Thus, one would expect to find a population of DNA molecules having one strand completely methylated (DNA synthesized before methionine starvation) while the other strand will have different lengths of unmethylated regions. Because all of the known restriction enzymes require doubly unmethylated DNA for cleavage it was difficult to account for the degradation of the DNA observed during methionine starvation. There were two possibilities to account for this degradation, (1) the E. coli 15 restriction enzyme can cleave DNA having only one unmethylated strand, or (2) doubly unmethylated DNA could arise by a mechanism other than initiation of new rounds of replication.

Evidence in favor of the second possibility was obtained in experiments in which the density label, 5-bromouracil, was substituted for thymine. Under conditions of methionine (or amino acid) starvation DNA containing 5-bromouracil in each strand was isolated. Recombination could account for this finding and this explanation is being pursued.

Significance to Biomedical Research and the Program of the Institute:

Findings pertaining to an elucidation of the mechanisms involved in the modification of nucleic acids are important as they may suggest possible means of therapeutic control of cellular growth.

Proposed Course:

1. Purification and characterization of the E. coli 15 restriction enzyme.
2. Further studies on S-adenosylmethionine synthesis and/or biological effects of S-adenosylmethionine analogues.

Publication:

Breitman, T. R., Maury, P. B., and Toal, J. N.: Loss of deoxyribonucleic acid-thymine during thymine starvation of Escherichia coli. J. Bact. 112: 646-648, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-936

Title: Recovery from effects of radiation or other initial treatment in terms of tolerance to subsequent treatments

Principal Investigator: Willie W. Smith

Section: Radiation Biology Section

Lab/Branch: Laboratory of Physiology

Goal: To understand the nature and persistence of damage from radiation and other therapeutic agents used singly and jointly in the treatment of cancer.

Approach:

Using the mouse as an experimental animal, the course of recovery from an initial irradiation is studied in terms of systems affected and residual dose-equivalent damage. The challenging or test exposures are given to unshielded animals for hematopoietic effects and to leg-shielded animals for evaluating the persistence of gastrointestinal damage. Studies are made of toxicity of radiation used jointly with other agents.

Progress:

The importance of identifying the system involved—hematopoietic or gastrointestinal—is emphasized in results of joint treatment with radiation and actinomycin D, vinblastine, or nitrogen mustard. In each case the nature of the damage and the time of recovery from a given agent is of critical importance.

Significance for Cancer Research (NCP Objective 6 Approach 1)

An understanding of the nature and duration of the damage to the host is important in establishing effective treatment programs.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-936

1. Laboratory of Physiology
2. Radiation Biology Section
3. Bethesda, Md.

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Recovery from effects of radiation or other initial treatment in terms of tolerance to subsequent treatments

Previous Serial Number: Same

Principal Investigator: Willie W. Smith

Man Years:

Total:	4
Professional:	1
Others:	3

Project Description:

Objectives: To identify and quantify effects of sublethal irradiation or chemotherapeutic treatments which alter tolerance to subsequent treatments.

Major Findings: In mice recovering from whole-body irradiation lethal to 1% of the number exposed, subsequent exposures reveal evidence of a persisting nonrecuperable damage. Although the nature of this damage is not known it is apparently limited to the hematopoietic system, for the lethal dose range is too low and survival too long to associate these deaths with acute intestinal injury. Since the initial exposure is sublethal, adequate hematopoietic function can be provided by shielding the legs during the second, challenging irradiation, thus making it possible to study gastrointestinal as distinct from hematopoietic recovery. Six hours after the initial, sublethal irradiation recovery amounted to 30% of the dose-equivalent damage in both systems; at 24 hours the hematopoietic system showed no further improvement while the gastrointestinal challenge indicated a 50% recovery; at 7 days the hematopoietic challenge indicated 70% recovery compared to 96% for the gastrointestinal challenge. At 3-4 weeks challenge of unshielded mice indicated 80% recovery, whereas the challenge of mice with legs shielded frequently indicated a slightly higher tolerance than in mice given no prior exposure.

Significance to Biomedical Research and the Program of the Institute:

A therapeutic program generally involves repeated treatments with one or more agents. We, and others, have shown toxic effects of joint radiation-actinomycin D or radiation-vinblastine to be strongly influenced by the order in which the treatments are given. An understanding of the nature of the damage to the host is especially important in establishing treatment programs which can be tolerated by the host.

Proposed Course of Project: I plan to continue studies of effects of treatments which are of interest in cancer therapy, particularly emphasizing their influence on tolerance to further treatment and possible differential effects on tumor and host.

Serial No. NCI-941

1. Laboratory of Physiology
2. Radiation Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunogenetic factors affecting bone marrow transplantation in irradiated mice: Modifying the functional capacity of bone marrow cells

Previous Serial Number: Same

Principal Investigator: Delta E. Uphoff

Man Years:

Total:	3.5
Professional:	1.0
Others:	2.5

Project Description:

Objectives: To facilitate bone marrow transplantation as an adjunct to toxic levels of either irradiation or chemotherapeutic agents in the treatment of cancer.

Methods Employed: Inbred mice, F₁ hybrids and especially derived substrains are exposed to toxic doses of X-irradiation and engrafted with bone marrow cells from different donors to test the effect of different degrees of genetic disparity on survival. Characteristic changes in the early survival patterns indicate changes in the ability of the marrow graft to respond to erythropoietic and antigenic stimuli while the long-term survival is affected by the graft-versus-host reaction and alterations in leukemogenesis. The focus of attention is on the response of the grafted marrow against the recipient (GVH reaction) as the primary obstacle in clinical marrow transplantation.

Major Findings: By employing bone marrow transplantation to investigate the antigen recognition mechanism methods of decreasing as well as increasing the GVH reaction were obtained. Although the object is to reduce the severity of the GVH reaction, it is equally important to determine which experimental procedures might have an adverse effect upon survival. Naturally-occurring adaptive mechanisms have been demonstrated to exist as maternal influences which affect the severity of the GVH reaction. Utilization of this mechanism could avoid prolonged use of immuno-suppressive drugs and thus prove beneficial.

In the previous report it was postulated that the mother is a potentially more compatible donor than the father. In fact, a significantly less severe GVH reaction followed the grafting of maternal marrow into lethally irradiated daughters. These results indicated that a reduced response to tissue alloantigens persisted in the mother long after parturition. In the case of lethally irradiated sons grafted with maternal marrow, however, a significantly more severe GVH reaction occurred indicating that the mother is sensitized to the antigens controlled by the Y-chromosome of male progeny. Two factors may be responsible for this anomaly. The time during development when antigens are expressed may be responsible for the failure to develop an unresponsive state since a short in vitro exposure to tissue antigens has been demonstrated to have a similar adverse effect when a longer exposure resulted in specific unresponsiveness. The effect is on the antigen recognition system and represents a cell surface interaction; consequently, the nature of the expression of the antigen on the surface of the cell is also a critical factor in the induction of the desired unresponsive state. The adverse effect on survival of sons grafted with maternal marrow occurs in both inbred strains and in hybrids, indicating that the sensitization is directed against the Y-antigen and does not represent an augmented response resulting from increased antigenicity, which would only occur in hybrids.

Maternal influences are vertically transmitted through the female line with no evidence of chromosomal or gene alterations. They appear to result from cell surface contact and are perpetuated by the fetal contact with the mammalian female. The ova-transfer substrains in which fertilized homozygous ova are transplanted to the uterus of a foster mother have been studied extensively in marrow transplantation experiments for evidence of maternal influences. Each substrain has one or more functional modification resulting from their ancestral contact with their foster mother. The types of modifications are a function of the genetic differences between the parental strain and the strain of the foster mother. In previous reports maternal influences reduced the phenotypic expression of tissue antigens and produced a reduced responsiveness toward tissue alloantigens. An ova-transfer substrain has now been found in which there is an increase in responsiveness transfer from the strain of the foster mother. In this case the increased response is directed against histocompatibility-2 antigens and the Y-antigens of male mice. While the marrow test system is sensitive enough to detect degrees of antigenicity and degrees of responsiveness, an increase in responsiveness would be difficult to attribute to a change in the phenotypic expression of immune response genes. Phenotypic changes invariably represent a reduction in the expression of a gene rather than an increase in the expression which would suggest gene duplication. Most test systems measure the presence or absence of a response rather than a quantitative difference in response as in the case of an increased gene dosage. The only other test used to measure responsiveness was that of skin allograft rejection. In comparing related substrains, there was no evidence of an increase in rejection rate suggestive of increased responsiveness. The resolution of this problem requires other more sensitive test systems than are presently available.

Significance to Biomedical Research and the Program of the Institute: Bone marrow transplantation, even with its shortcomings, has supplied a method for testing maternal influences and made possible experimental manipulation of the antigen recognition system. The maternal influence is the expression of a basic adaptive mechanism which is assumed to have evolved through survival pressures associated with preventing the rejection of the hybrid fetus by the mammalian female. Similarly, neoplasms with demonstrable tumor antigens grow progressively following a similar adaptive process. The application of this basic mechanism to other biological phenomena was more extensively considered in a recent review (Biomedicine 18: 13-22, 1973).

Proposed Course of Project: No change in the course of this investigation is anticipated as long as new aspects of the antigen recognition system are revealed. Other aspects of this project reported in previous years are being continued. The project will be expanded to include consideration of the mechanism by which modification may be induced as soon as new approaches to this problem are developed.

Publications:

Uphoff, D. E.: Maternal influence on the immune response. Biomedicine 18: 13-22, 1973.

Uphoff, D. E.: Survival of irradiated hybrid female mice inoculated with maternal bone marrow: A preliminary report. J. Natl. Cancer Inst. 50: 263-265, 1973.

Uphoff, D. E.: Maternal control of the immune response. Transplant. Proc. 5: 197-199, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 941

Title: Immunogenetic factors affecting bone marrow transplantation in irradiated mice: Modifying the functional capacity of bone marrow cells.

Principal Investigator: Delta E. Uphoff

Section: Radiation Biology

Lab/Branch: Laboratory of Physiology

Goal: To facilitate bone marrow replacement as an adjunct to radiation and chemotherapy for the treatment of cancer.

Approach: Classical immunologic methods have failed to produce a universally effective method of avoiding the immune response of the grafted marrow against the recipient or other equally devastating side effects. Although experimental and clinical results are unpredictable, sufficient success has been achieved to indicate that adverse side-effects are avoidable. Basic experiments on mice indicate that marrow stem cells have a functional flexibility characteristic of embryonic cells and are consequently subject to environmental conditioning. The next step was to determine which environmental factors influenced the functional capacity of the marrow stem cell and to determine the extent to which their function could be experimentally manipulated. In close correlation was the use of marrow transplantation to investigate the influence the maternal environment exerts on the expression of tissue antigens and on the ability of mice to respond to specific antigenic stimuli. Since maternal conditioning has resulted in stable mixed chimerism in the case of nonidentical twins it should be possible to produce compatible chimerism by a similar artificially induced conditioning.

Progress: Maternal influences affect the antigen recognition system, i.e. the maternal environment qualitatively reduces the phenotypic expression of tissue antigens while the existence of a lower level of antigenicity results in a failure to recognize and respond to these antigens as foreign. This equilibrium results in unresponsiveness but only for qualitatively modified antigens and not for fully expressed antigens. Experimental conditioning was used to avoid the graft-versus-host reaction. However, there are other anomalies that require investigation since multiparous females may be unresponsive to tissue alloantigen of their daughters but sensitized to antigens controlled by Y-chromosomes of their sons. The way antigens are presented to the antigen recognition system may be a key factor in separating unresponsiveness from sensitization.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Bone marrow replacement would facilitate the use of high levels of irradiation and chemotherapeutic agents that produce toxic effects to the hematopoietic system during the treatment of leukemia and other neoplasms. It would also aid in management of aplasia, congenital erythrocyte anomalies and immunodeficiency diseases.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-942

1. Laboratory of Physiology
2. Radiation Biology Section
3. Bethesda, Md.

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Effects of Ionizing Radiation on Nucleic Acids, Proteins and Their Constituents: Radioprotective and Radiosensitizing Agents

Previous Serial Number: Same

Principal Investigator: Peter Riesz

Other Investigators: S. Kominami, Visiting Fellow
E. Rotlevi, Visiting Fellow

Man Years:

Total:	5
Professional:	3
Others:	2

Project Description:

Objectives:

The effects of ionizing and ultraviolet radiation on biological macromolecules and their constituents are being investigated. For reproductive death, DNA is the target molecule in viruses and is at least a part of the target molecule in bacteria and mammalian cells. Radiation damage to DNA is produced by the "direct effect" through the formation of radical ions, electrons, excited states and neutral free radicals or by the "indirect effect" where radical species formed in the surrounding medium by radiation react with DNA. In the case of water, these species are hydrated electrons, hydrogen atoms and hydroxyl radicals. In the case of radiation damage to the chromosome, radicals formed in the nucleohistone may cause damage to the DNA.

In the chain of events that leads to loss of biological activity, free radicals play an important role. Thus, for example, when biologically active DNA of bacteriophage $\phi \times 174$ is irradiated in the dry state with γ -rays under vacuum, cysteamine protects to the same extent (by about a factor of 1.6) against inactivation and the production of free radicals. We have found recently that the addition of

certain metal ions to DNA in the ratio of 1 metal ion per 10 nucleotides, can reduce the radical yield by a factor of about 10.

An understanding of the mechanisms by which ionizing radiation brings about the loss of biological activity of these macromolecules is likely to help in the development of new methods for altering the efficiency of cell killing with possible benefits to radiation therapy.

Methods Employed:

When dry proteins or nucleic acids are irradiated with gamma rays or ultraviolet radiation in the absence of air (at 10^{-4} mm pressure), trapped free radicals are generated. Subsequent exposure to tritiated hydrogen sulfide or hydrogen iodide leads to the formation of carbon-tritium bonds. After removal of exchangeable tritium the protein is hydrolyzed, the amino acids are separated on an automatic amino acid analyzer and the tritium content of each amino acid is determined by means of an associated flow counter. It has been shown that the tritium distribution so obtained corresponds closely to the free radical distribution at the time of exposure to the tritiated free radical interceptor.

Trapped radicals in lyophilized proteins and nucleic acids were produced by:

(a) Gamma-radiolysis using a Co^{60} source.

(b) Photolysis with 2537 Å light using a low pressure Mercury arc. A PCQ-X1 Photochemical Lamp with 4 circular lamps was used; 80-90% of the total radiation is at 2537 Å.

(c) Electrical discharge was generated by a high frequency induction coil (Tesla Type) adjusted for maximum voltage (40-50 kv).

Electron Spin Resonance studies were carried out at Walter Reed Army Medical Center using the Varian E-9 ESR Spectrometer located in the laboratory of Dr. E. S. Copeland.

Proteins and DNA were tritium labeled on exchangeable sites by exposing the dried materials to tritiated water vapor (5 curie/gm) at 20 torr for 10 minutes in a high vacuum system located in a hood in the Isotope Laboratory (Bldg. 21).

Major Findings:1. Effects of Metal Ions on Radical Formation in γ -Irradiated DNA in the Solid State (with E. Rotlevi and S. Kominami)

The effects of metal ions on the formation of radicals produced by γ -radiolysis of DNA in vacuo were studied by ESR and hydrogen-transfer measurements. At metal to nucleotide ratios of 1:10 a 3- to 10-fold decrease of the ESR signal due to carbon was observed for transition metals such as Cr^{3+} , Fe^{3+} , Fe^{2+} , Ni^{2+} and Co^{2+} and smaller decreases (by 25-40%) for paramagnetic rare earths (Eu^{3+} , Yb^{3+} and Sm^{3+}). These effects were independent of dose and temperature (77-298°K). The diamagnetic ions Mg^{2+} , Zn^{2+} , Cd^{2+} , Ag^{+} , La^{3+} and Lu^{3+} had no effect; however, Ce^{4+} , Pb^{2+} , Sn^{4+} and Hg^{2+} decreased the radical yield by 10-60%. Only those diamagnetic ions which possess more than one stable oxidation state have appreciable effects. At metal to nucleotide ratios of 1:100 the radical yield was decreased twofold for Cr^{3+} , Cu^{2+} , and Fe^{2+} ; about 30% for Mn^{2+} , Ni^{2+} and Fe^{3+} ; and < 10% for Co^{2+} .

Substantial progress was made in three other areas:

(a) Experiments were undertaken to rule out a possible alternative explanation of the decreased electron spin resonance signals in the presence of paramagnetic ions. It was recently shown by Leigh [J. Chem. Phys. 52, 2608 (1970)] that under certain circumstances a decreased amplitude of the ESR signal without line broadening can occur as a result of spin-spin interaction between carbon radicals and paramagnetic ions. Microwave power saturation studies for the carbon radicals in DNA and DNA-metal complexes were carried out to measure the relaxation times ($T_1 \cdot T_2$). From these, the relative strength of the spin-spin interaction can be estimated. The strongest spin-spin interaction occurs in DNA- Co^{2+} for both the (1:100) and (1:10) complexes, and the DNA- Ni^{2+} (1:100) complex has no significant spin-spin interaction. The spin-spin interactions of the various DNA-metal complexes do not correlate with the observed decrease of ESR signals, and hence the decrease of the ESR signal cannot be explained by spin-spin interactions. This conclusion was supported by theoretical calculations and by the observation of a non-linear dependence of the relative radical yield on nickel concentration.

(b) The study was extended by carrying out the γ -radiolysis at 77°K. The effects of CoSO_4 , NiSO_4 , and EuCl_3 in decreasing the radical yield were approximately the same at 77°K as at room temperature. This result indicates that the metal ions act at a very early stage by preventing the formation of precursors (primarily cationic and anionic radicals, and to a lesser extent, excited states) of the neutral radicals. Moreover, there was no change in the relative radical yield after warming to room temperature following γ -radiolysis at 77°K. This result indicates that these metal ions do not quench triplet states produced by ion recombination and do not

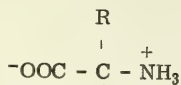
interfere with the transformation of primary to secondary radicals by redox reactions with neutral radicals.

(c) Ni^{2+} ions, present in a ratio of one ion per 10 monomers, decrease the radical yield in DNA, polyadenylic acid, adenine, dextran, poly-DL-alanine and poly-L-glutamic acid by approximately 60-70%. Hence, it appears likely that the radiation chemistry of a great variety of organic solids can be modified by introducing small amounts of metal ions to form molecular mixtures. A special property of a particular organic solid (such as base stacking in DNA) is unlikely to play a dominant role in the mechanism by which metal ions exert their effects.

2. Effects of Metal Ions on the γ -Radiolysis of Polycrystalline Djenkolic Acid (with S. Kominami)

This compound is of special interest because it allows one to separate the effects of metal ions on (1) carbon radical formation, (2) thermal carbon radical decay, (3) transfer of unpaired spins from carbon to sulfur, and (4) the radiation-induced destruction of sulfur radicals. All of these processes occur in γ -irradiated proteins and the effect of metals on them is difficult to disentangle. It was found that Co^{2+} , Fe^{2+} , Ni^{2+} , Ni^{2+} -EDTA and Ce^{4+} decrease the initial radical yield by about 50%, while Cd^{2+} has no effect. A detailed kinetic study of the radical decay at room temperature showed that carbon radical decay could be quantitatively explained by assuming two different radical species. Radical species A transforms slowly to sulfur radicals by a first-order process while radical species B disappears by a second-order recombination (or disproportionation) process.

Radicals A and B have the typical "doublet" structure and are alpha carbon radicals



but are presumably found in different microenvironments in the crystal. There is no observable change in the carbon ESR signal during radical decay.

Ni^{2+} (1:10) and Ni^{2+} -EDTA (1:10) bring about a 2- and a 4-fold increase, respectively, of the second order recombination rate constant. Radical recombination may be due either to a vacancy-controlled bulk diffusion in the solid or to a series of successive hydrogen atom abstraction reactions. If the first hypothesis is correct, then the effect of the metal ions can be understood in terms of an increased number of lattice defects in the presence of metal ions. There is no effect of metal ion on the unimolecular conversion of carbon radicals to sulfur radicals at room temperature.

After 380 hours at room temperature the second order radical combination is essentially complete. Heating to 110°C for 5 hours converts all remaining carbon radicals to sulfur radicals. This process is not affected by any metal except Ni^{2+} . The radiation-induced destruction of the sulfur radicals, produced by heating to 110°C, can then be investigated by γ -radiolysis at 77°K. This radiation-induced destruction, which is the sum of two first-order processes and proceeds with negligible activation energy, is suppressed by the presence of Ni^{2+} ions.

Significance to Biomedical Research and the Program of the Institute:

Studies of the effects of ionizing radiation are of importance in relation to (1) radiation therapy, (2) carcinogenesis, (3) stability of the genetic pool, (4) the suppression of the immune mechanism, and (5) aging. The effects of ionizing radiation on nucleic acids are being studied in order to understand the nature of radiobiological death in normal and tumor cells. The addition of radioprotective and radiosensitizing agents is being investigated so that a therapeutic advantage may be gained.

Proposed Course of Project: To continue studies on the effects of ionizing radiation on macromolecules of biological importance. The mechanism of radioprotective and radiosensitizing agents will be investigated.

Honors:

Invited Lecture: Effects of Metal Ions on Radical Formation in γ -Irradiated DNA in the Solid State. International Conference on Electron Spin Resonance and Nuclear Magnetic Resonance in Biology and Medicine, The New York Academy of Sciences, December 1972.

Publications:

Riesz, P. and Smitherman, T. C.: Radiation-induced hydrogen transfer in nucleic acids and proteins in the solid state. Israel J. of Chem. (in press).

Rotlevi, E., Moss, H. M., Kominami, S., and Riesz, P.: Effects of Metal Ions on Radical Formation in γ -Irradiated DNA in the Solid State. Ann. NY Acad. Sci. (accepted for publication).

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-942

Title: Effects of Ionizing Radiation on Nucleic Acids, Proteins and Their
Constituents: Radioprotective and Radiosensitizing Agents

Principal Investigator: Peter Riesz

Section: Radiation Biology

Lab/Branch: Laboratory of Physiology

Goal: To investigate and develop new radioprotective and radiosensitizing agents in order to obtain a therapeutic advantage in radiation therapy.

Approach: In the chain of events that leads to loss of biological activity of DNA and proteins after exposure to γ -radiation, free radicals play an important role. The effects of radioprotective and radiosensitizing agents on the production of free radicals in DNA, proteins and their constituents are being investigated by means of electron spin resonance measurements and by the radiation-induced transfer of tritium from exchangeable positions (those hydrogens which are bound to nitrogen and oxygen) to carbon-bound positions.

These studies will be supplemented by comparisons of the effects of the same additives on the production of single and double chain breaks in nucleic acids and main chain breaks in proteins as measured by the sedimentation equilibrium method. Since the interior cells in tumors are often in an anoxic state (which decreases radiation sensitivity) while the surrounding tissue is well oxygenated, attention will be focused on protective agents which act in the presence of oxygen and sensitizers which act only under anoxic conditions.

Progress: The effects of metal ions and complexes on the formation of radicals produced by γ -radiolysis of DNA were studied by ESR and hydrogen transfer measurements. At metal to nucleotide ratios of 1:10, a 3- to 10-fold decrease of the ESR signal, due to carbon, was observed for certain transition metal ions and their complexes. Even at metal to nucleotide ratios of 1:100, the radical yield was decreased 2-fold for certain metal ions. It appears that charge and/or energy migrate over large distances in γ -irradiated DNA. Studies of γ -irradiated djenkolic acid allows the separation of the effects of metal ions on a) carbon radical formation, b) thermal radical decay, c) conversion of carbon to sulfur radicals and d) the radiation-induced destruction of the sulfur radicals.

Significance for Cancer Research (NCP Objective 6 Approach 1)

The goal of this project is to increase the differential radiation sensitivity of neoplastic cell populations by means of radiation-protective and radiosensitizing agents. Studies of the effects of ionizing radiation on DNA are also related to understanding radiation-induced carcinogenesis.

Level of Effort: Professional Man-Years: 3

SUMMARY STATEMENT
ANNUAL REPORT

July 1, 1972 through June 30, 1973

General Summary of the Work in the LABORATORY OF TUMOR PATHOPHYSIOLOGY

The central goal of the laboratory is to increase our understanding of the regression mechanism in hormone dependent mammary tumors. The assumption is that this knowledge will enhance our capability of controlling the growth and metastasis of these tumors.

In the first part of 1972, the physiopathological characterization of our experimental models, primary DMBA induced and MTW9 transplanted mammary tumors of SD and F/W female rats was completed. The conclusion was reached that in these models, which resemble closely the human disease, regression consisted of a coordinated sequence of events which occurred in the whole tumor and resulted in the destruction of single cells apparently at random. Vascular, interstitial and cellular compartments, blood supply and consumption of basic metabolites, such as oxygen and glucose, were similar in growing and regressing tumors on a tissue weight basis (1,3). The increment of activities of 6 lysosomal enzymes, which was known to occur during regression, was found to involve only the neoplastic cells, not the pericellular fluid. There was no indication that during regression lysosomal enzymes were "leaking" as active enzymes into the interstitial spaces of the tissue (2). The increase in lysosomal enzyme activities was due to an increase in the number of protein molecules (4) and the demonstration of this event was made possible by a modification of a radioimmuno assay (5). Among the lysosomal enzymes, RNase activity was found sharply increased very early in regression, i.e. 6 hrs. after hormonal deprivation of the host. On the assumption that this increase could be related to the initiation of regression, tumor RNase was purified to electrophoretic homogeneity and then crystallized from ammonium sulfate solutions. Anti-RNase serum was produced in rabbits and used to quantitate the enzyme during tumor regression, utilizing the modified radioimmuno assay previously mentioned (5). Within a few hours after hormonal deprivation of the host both synthesis and degradation of RNase increased, synthesis more than degradation with a net increment of total enzyme molecules roughly paralleling the increase in activity (5,6). Cycloheximide given at a proper dosage was able to block, almost totally, incorporation of labelled precursors into RNA and proteins. Under these conditions the increment of lysosomal enzymes did not occur but regression continued undisturbed. We assume that hormonal deprivation of the host elicits tumor cell destruction which is rapidly followed by cannibalism of cell fragments by the surviving population. Increment of lysosomal enzyme activities and quantities probably relates to the second event. On the assumption that cycloheximide treatment eliminates the interference of the metabolic processes mobilized by cannibalism, investigation of tumor regression is now carried on by Drs. Cho-Chung and Gullino using tumors from hosts treated with cycloheximide.

Mammary tumor regression is also studied by Dr. Knazek following another approach. Drs. Knazek and Gullino have developed a vascular network constituted by artificial capillaries and able to sustain growth of neoplastic cells to a density comparable to that of a tumor in vivo, i.e. about 100×10^6 cells per cm^5 (8). Cells of a human choriocarcinoma living at such densities were

able to produce gonadotropins at a per cell rate about 10 fold higher than in monolayer culture (9). Attempts at reproducing the regression in vitro are now being made using epithelial cells of mammary tumors grown to tissue density as organoid structures in the artificial capillary network. It is believed that the study of a uniform neoplastic cell population should favor the analysis of the events which trigger regression.

Another approach to the study of tumor regression is followed by Dr. Gimbrone. It is known that neoplastic cells grow to form a tumor mass only if they are able to produce a tumor angiogenesis factor which elicits new formation of vessels by the host (10). During the past year, Dr. Gimbrone has isolated endothelial cells from human umbilical cord veins and cultivated them as monolayers. He now has the possibility of determining whether the angiogenesis factor is able to stimulate mitoses in these cells. This assay appears to be an indispensable tool for testing the activity and thus for improving the purification and characterization of the angiogenesis factor. The distant goal of this project is the finding of an anti-angiogenesis preparation to be utilized in the prevention of metastasis. In a second project, Dr. Gimbrone is exploiting the ability of neoplastic cells to induce angiogenesis for diagnostic purposes. Hyperplastic alveolar nodules of the mouse mammary gland are removed at various intervals of their transformation into the carcinomatous form and implanted in a pouch of the rabbit cornea. The new formation of vessels coming from the pericorneal region is easily detected in this system and the question we want to answer is whether the ability to elicit neovascularization is a sign which distinguishes the hyperplastic from the neoplastic tissue. Human material will also be tested as soon as the assay is fully operative.

Dr. Butler studies the regression process from another angle. It is well known that primary mammary tumors are easily removed by surgical procedures but the metastases kill the patient. Dr. Butler looks for an answer to the following questions: What is the number of neoplastic cells that a mammary tumor discharges into the blood stream per hour? Is there a change in this number or in the type of cells released when the tumor regresses or when it metastasizes? In the past years, Dr. Gullino and co-workers have developed an experimental system in which the blood of the whole tumor is collected by a single vein. Dr. Butler utilizes these preparations to collect efferent blood from primary and transplanted mammary tumors of rats which mimic the human disease. In the past year, he has ascertained that neoplastic cells released in blood by these tumors cannot be morphologically distinguished from monocytes. He proceeded then to prepare an antiserum using the isolated neoplastic cells as antigens. This antiserum is now sufficiently specific to permit detection of neoplastic cells using fluorescein as an indicator. It is likely that within a few months, Dr. Butler will be able to give a documented answer to the above questions.

REFERENCES

1. Gullino, P.M., Grantham, F.H., Losonczy, I. and Berghoffer, B.: Mammary tumor regression: I. Physiopathological characteristics of hormone dependent tissue. J.Nat. Cancer Inst., 49: 1333-1347, 1972.

2. Gullino, P.M. and Lanzerotti, R.H.: Mammary tumor regression: II. Autophagy of neoplastic cells. J.Nat. Cancer Inst. 49: 1349-1356, 1972.
3. Gullino, P.M., Grantham, F.H., Losonczy, I. and Berghoffer, B.: Mammary tumor regression: III. Uptake and loss of substrates by regressing tumors. J.Nat. Cancer Inst. 49: 1675-1684, 1972.
4. Lanzerotti, R.H. and Gullino, P.M.: Activities and quantities of lysosomal enzymes during mammary tumor regression. Cancer Res. 32: 2679-2685, 1972.
5. Lanzerotti, R.H. and Gullino, P.M.: Immunochemical quantitation of enzymes using multispecific antisera. Anal. Biochem. 50: 344-353, 1972.
6. Cho-Chung, Y.S. and Gullino, P.M.: Mammary Tumor Regression: V. Role of Acid Ribonuclease and Cathepsin. J. Biol. Chem., In Press, 1973.
7. Cho-Chung, Y.S. and Gullino, P.M.: Mammary Tumor Regression VI. Synthesis and Degradation of Acid Ribonuclease. J. Biol. Chem., In Press, 1973.
8. Knazek, R.A., Gullino, P.M., Kohler, P.O. and Dedrick, R.L.: Cell culture on artificial capillaries: an approach to tissue growth in vitro. Science 178: 65-67, 1972.
9. Knazek, R.A. and Gullino, P.M.: Effect of cell density on gonadotropin production by a human choriocarcinoma cell line. In Preparation.
10. Gimbrone, M.A. et al.: Tumor dormancy in vivo by prevention of neovascularization. J. Exp. Med. 136: 261-276, 1972.

Serial No. NCI-368

1. Laboratory of Tumor Pathophysiology
2. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Studies on the Mechanisms of Mammary Tumor Regression.

Previous Serial No.: NCI-368

Principal Investigator: Dr. P.M. Gullino

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 3

Professional: 1

Other: 2

Project Description:

Objectives: To understand the mechanism of regression in hormone-responsive mammary tumors.

Methods Employed: Transplantable MTW9 and primary 7-12 DMBA-induced mammary tumors are used as in the past, and a new model has been added, primary mammary tumors induced by methylnitrosamine in Buff/N ♀ rats. Primary methyl cholanthrene-induced mammary tumors have been discarded because of unreliable growth behavior.

Tumor regression is induced by castration ± adrenalectomy and/or removal of pituitary hormones. Procedures developed in our laboratory, such as tissue-isolated preparations using primary or transplantable tumors, or sampling of tumor interstitial fluid by micropore chambers, and standard physiological techniques such as continuous intra-aortic infusion of solutions, cannulation of tumor vessels etc., are being applied to the study of tumor regression.

Major Findings:

1) Publication has been completed of a first group of observations which indicate that regression of hormone-dependent mammary tumors is an event triggered apparently at random within each cell at various times after hormone removal while basic physiological parameters of the tissue, such as blood supply, oxygen removal ratio, glucose consumption, etc., remain within physiological limits. The role of RNA in the sequence of events related to the triggering of regression has been studied. Two major observations have been made: a) An increase of RNA synthesis occurs in the neoplastic tissue within a few hours after hormonal deprivation of the host, b) The synthesis involves all RNA molecular species without any particular trend, with the possible exception of chromosomal RNA. Thus in the hormone-dependent tumors, the same sequence of events can be observed when the

tissue is either stimulated by or deprived of the hormone: RNA synthesis occurs first, followed by protein and lipid syntheses.

2) The burst of RNA synthesis, which characterizes the initiation of regression, may be an essential part of the mechanism triggering regression but it could also be the consequence of an increase in metabolic activities related to cannibalism of cell fragments by non-regressing tumor cells. Attempts to dissociate the two phenomena are under way, following two approaches: a) block of RNA and protein synthesis induced by cannibalism and analysis of tumor regression, and b) preparation of an epithelial cell culture which regresses in the same way as the whole tumor. We have learned to block RNA and protein syntheses and arrest tumor growth; the events conducive to regression are being studied in this system. We have also obtained the ascites form of at least one hormone-dependent mammary tumor and we are planning to isolate selectively epithelium from fibroblastic cells following the micropore chamber method previously reported. In this way, we hope to develop an in vitro preparation capable of mimicing the in vivo regression process.

Significance to Cancer Research: Clarification of the events determining growth arrest and regression of mammary tumors in experimental models will contribute to the understanding of the human disease.

Proposed Course of Research: To continue the study of the mechanism of mammary tumor regression.

Honors and Awards: Golden Ambrosino, presented in Milan, Italy, May, 1972; Knazek, R.A., Gullino, P.M., Dedrick, R.L. and Kidwell, W.R.: U.S. Patent Application Serial No. 254,678. "Cell culture on semi-permeable tubular membranes".

Publications:

1. Gullino, P.M., Grantham, F.H., Losonczy, I. and Berghoffer, B.: Mammary tumor regression: I. Physiopathological characteristics of hormone dependent tissue. J.Nat.Cancer Inst. 49: 1333-1348, 1972.
2. Gullino, P.M., and Lanzerotti, R.H.: Mammary tumor regression: II. Autophagy of neoplastic cells. J.Nat. Cancer Inst. 49: 1349-1356, 1972.
3. Gullino, P.M., Grantham, F.H., Losonczy, I. and Berghoffer, B.: Mammary tumor regression: III. Uptake and loss of substrates by regressing tumors. J.Nat.Cancer Inst. 49: 1675-1684, 1972.
4. Lanzerotti, R.H. and Gullino, P.M.: Activities and quantities of lysosomal enzymes during mammary tumor regression. Cancer Res. 32: 2679-2685, 1972.
5. Lanzerotti, R.H. and Gullino, P.M.: Immunochemical quantitation of enzymes using multispecific antisera. Anal.Biochem. 50: 344-353, 1972.
6. Grantham, F.H., Hill, D.H. and Gullino, P.M.: Primary mammary tumors connected to the host by a single artery and vein. J.Nat.Cancer Inst. 50: 1381-1383, 1972.

7. Gullino, P.M.: Regression of solid tumors. In Garattini, S. and Franchi, G. (Eds.): Chemotherapy of Cancer Dissemination and Metastasis. New York, Raven Press, 1973, In Press.
8. Knazek, R.A. and Gullino, P.M.: Artificial capillaries: an approach to tissue growth in vitro. In Kruse, P.F. and Patterson, M.K. (Eds.): Tissue Culture: Methods and Applications. New York, Academic Press, 1973, In Press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-368

Title: Studies on the Mechanisms of Mammary Tumor Regression

Principal Investigator: P. M. Gullino

Section:

Lab/Branch: Laboratory of Tumor Pathophysiology

Goal: Characterization of the events occurring during mammary tumor regression.

Approach: Mammary tumors of rodents, both transplantable and carcinogen-induced, have been selected to mimic the hormone responsiveness of the human mammary tumors. A new method for obtaining primary mammary tumors connected to the host only by one vein and one artery was developed. Thus, sampling of afferent and efferent tumor blood was possible during regression; and studies on blood supply, oxygen removal ratios, glucose consumption, lactate production, loss and uptake of various metabolites by the regressing tumor were completed and a definition of the regression process in physiopathological terms was established. The role of RNA in the sequence of events related to the triggering of regression was studied next.

Progress: Two major observations were made: a) Within a few hours after hormonal deprivation of the host a burst of RNA synthesis occurs in the tumor which will regress and b) new synthesis involves all RNA molecular species without a particular trend with the possible exception of chromosomal RNA. Since destruction of a cell induces cannibalism in adjacent cells, we are now trying to understand whether the increase of RNA synthesis is an indispensable event in the triggering mechanism of regression or a secondary effect due to metabolic stimulation following cannibalism.

Significance for Cancer Research (NCP Objective 6 Approach 3): If one knows the sequence of events recurring during regression of hormone-dependent mammary tumors, one may learn to trigger the occurrence of these events and to induce regression in all types of mammary tumors.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-5216

1. Laboratory of Tumor Pathophysiology
2. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Role of Lysosomal enzymes in hormone-dependent rat mammary tumor regression.

Previous Serial No.: NCI-5216

Principal Investigator: Dr. Yoon Sang Cho-Chung

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 2 1/2

Professional: 1

Other: 1 1/2

Project Description:

Objectives: Previously, our studies demonstrated a significant increase in six lysosomal enzyme activities during the early phase of tumor regression. Further studies were undertaken to assess whether the increased activity of lysosomal enzymes is due to the increased quantities of the enzymes and to determine if the accumulation of the enzyme is the result of change in the rate of synthesis or degradation or both parameters.

Methods: Primary 7-12 DMBA induced and transplantable MW9 rat mammary tumors were used. Both tumors regress when appropriate hormonal stimulus is removed from the host. Correlation of the activity to the quantity of β -galactosidase and β -glucuronidase was determined by a histochemical modification of the single radial immunodiffusion method. The isolation of acid RNase in a homogeneous state permitted the production of specific anti-acid RNase antiserum and the subsequent quantitation of the enzyme. The rates of synthesis and degradation of acid RNase in growing and regressing tumors were determined by the immunological precipitation of the enzyme with anti-acid RNase antiserum following in vivo labeling.

Major Findings:

- 1) Regressing tumors contained larger quantities of β -galactosidase and β -glucuronidase, and the increased amounts closely corresponded to the increased activities.
- 2) Burst of acid RNase activity as early as 6 hours after hormone removal paralleled the increased quantity of enzyme.
- 3) Increased acid RNase synthesis during tumor regression was demonstrated in-

directly by measuring the decrease in specific activity of the enzyme labeled with ^{14}C -glucose during tumor growth.

4) Pulsed H^3 -leucine incorporation directly into acid RNase demonstrated a significantly increased rate of synthesis of the enzyme which peaked at 12 hours after hormone removal.

5) The rate of radioactivity loss from the pre-labeled acid RNase in vivo demonstrated that the degradation rate of the enzyme increased during the initial phase of tumor regression but later approached the rate present in the growing tumors.

6) Determination of rate constants of both synthesis and degradation of acid RNase indicated that rapid turnover (synthesis and degradation) of acid RNase occurs during the initial phase of tumor regression with accumulation of the enzyme as the end result.

Significance to Cancer Research: The studies contribute to the understanding of biochemical events underlying the regression of experimental mammary tumors which mimic human breast cancer. Whether or not the increased turnover of acid RNase demonstrated during the initial phase of tumor regression has any correlation with initiation or triggering of the regression process can not be answered at this time.

Proposed Course of Research: To extend the studies on the mechanism of mammary tumor regression, the following proposal is made; i) establish the causal relation of acid RNase to tumor regression, ii) test the role of cyclic adenosine-3',5'-monophosphate (hormonal mediator or second messenger) in the regression process, iii) test the order of intracellular component degradation during tumor regression by mapping the cellular localization of lysosomal enzyme (acid RNase) and non-lysosomal enzyme (glucose-6-phosphate dehydrogenase) by the use of immunological analyses.

Honors and Awards: None

Publications:

1. Cho-Chung, Y.S. and Gullino, P.M.: Mammary Tumor Regression: V. Role of Acid Ribonuclease and Cathepsin. J. Biol. Chem., In Press.
2. Cho-Chung, Y.S. and Gullino, P.M.: Mammary Tumor Regression: VI. Synthesis and Degradation of Acid Ribonuclease. J. Biol. Chem., In Press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5216

Title: Role of Lysosomal enzymes in hormone-dependent rat mammary tumor regression.

Principal Investigator: Yoon Sang Cho-Chung

Section:

Lab/Branch: Laboratory of Tumor Pathophysiology

Goal: Investigate the causal relation of lysosomal enzymes to the regression of experimental mammary tumors which mimic many biological properties of human breast cancer.

Approach: 1) The temporal relationship of activities and quantities of lysosomal enzymes with tumor regression has been assessed. 2) The rate of synthesis and degradation of lysosomal enzymes has been determined by immunological analysis after in vivo labeling. 3) The mapping of cellular localization of acid RNase and glucose-6-phosphate dehydrogenase is being carried out in an attempt to detect which of the intracellular components are first involved in degradation. Detection of the topographical initiation of the regression process in the cell might help to clarify the triggering mechanism.

Progress: 1) The increment of lysosomal enzyme activities occurs relatively late as compared with the first signs of reduction in tumor volume. We believe that the increased activity is probably not a cause but rather a consequence of the regression process, probably related to elimination of catabolites. 2) Initiation of regression is followed by a sharp increase in the rate of both synthesis and degradation of lysosomal enzymes. Increased enzymatic activity is due to accumulation of new enzyme molecules because as regression starts the rate of synthesis becomes higher than that of destruction. 3) Purification of acid RNase and production of the antiserum has been completed. Purification of G6PD and isoenzymes is proceeding and an antiserum preparation is expected within a few months.

Significance for Cancer Research (NCP Objective 6 Approach 3): Studies of lysosomal enzymes in experimental regressing tumors could provide an insight into the mechanisms of tumor regression. The understanding of these regression mechanisms could, then, lead to the development of ways of inducing regression in hormone-independent tumors.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-5219

1. Laboratory of Tumor Pathophysiology
2. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: In vitro culture of solid tumors.

Previous Serial No.: NCI-5219

Principal Investigator: Dr. R. A. Knazek

Other Investigators: P.M.Gullino and P.O.Kohler

Cooperating Units: Endocrine division of NICHD

Man Years:

Total: 1 1/2 + 1 (Stride)

Professional: 1

Other: 1/2 + 1 (Stride)

Project Description:

Objectives: Continued development of the technique of cell culture on artificial capillaries in vitro.

Methods: Artificial capillary beds, constructed by sealing a mixture of silicone and polysulfone capillaries in a glass tube (0.8 x 9 cm), were perfused with sterile nutrient medium at 37°C. Cells injected into the extracapillary space were nourished by substances diffusing from the perfusate through the capillary walls, while products diffused away from the cells and into the perfusate. The medium was replaced periodically.

Major Findings: An established choriocarcinoma cell line grew to near-tissue density in between the capillaries and continued to secrete human chorionic gonadotropin at rates approximately eleven times greater than those of the same cells growing in standard monolayer forms.

Significance to Cancer Research: This technique allows established lines of cells to grow to near-tissue density in vitro. Study of the hormone levels in the perfusate indicate that cells do behave differently in these tissue-like densities, probably in a manner more similar to their behavior in vivo.

Large numbers of cells can, therefore, be grown in a relatively simple and small apparatus. The responses of cells grown and maintained in this manner to biochemical and pharmacological alterations of nutrient medium will probably most closely simulate their responses in vivo but without the complicating and uncontrollable in vivo variables.

Proposed Course of Research: Growth and regression of human and rat mammary

carcinomas will be studied using the method of cell culture on artificial capillaries.

Honors and Awards: Knazek, R.A., Gullino, P.M., Dedrick, R.L. and Kidwell, W.R., U.S. Patent Application Serial No. 254,678. "Cell Culture on Semi-Permeable Tubular Membranes."

Publications:

1. Knazek, R.A., Gullino, P.M., Kohler, P.O. and Dedrick, R.L., Cell Culture on Artificial Capillaries: An Approach to Tissue Growth in Vitro. Science 178: 65-67, 1972.
2. Knazek, R.A. and Gullino, P.M.: Artificial Capillaries: An Approach to Tissue Growth in Vitro. In Kruse, P.F. and Patterson, M.K. (Eds.): Tissue Culture: Methods and Applications. New York, Academic Press, 1973, In Press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5219

Title: In vitro culture of solid tumors.

Principal Investigator: R.A. Knazek

Section:

Lab/Branch: Laboratory of Tumor Pathophysiology

Goal: Continued development of the technique of cell culture on artificial capillaries in vitro.

Approach: An artificial capillary network has been assembled utilizing commercially available products. The material which constitutes these capillaries modulates their physiological properties. The success in growing cells depends on the combination of different types of capillaries so that the culture is ensured a proper exchange of gases, metabolites and catabolites.

Progress: Human choriocarcinoma cells grown on closely juxtaposed artificial capillaries perfused with nutrient medium have attained cell densities approaching those of tissues, approximately 100×10^6 cells per cc. To our knowledge, this had never been accomplished before. Behavior of the cells grown in this manner has been shown to differ from that observed in monolayer culture as indicated by an eleven-fold increase in the rate of human chorionic gonadotropin secretion by each cell.

Significance for Cancer Research (NCP Objective 4 Approach 1): Established cell lines growing to tissue-like densities in vitro afford a controlled and efficient system for studying cell function under conditions closely simulating those in vivo. The demonstration that a cell in capillary culture units has a rate of gonadotropic production eleven-fold higher than a cell in monolayer, indicates that cell cultivation to tissue density adds a new dimension to the study of solid tumors.

Level of Effort: Professional Man-Years: 1

Serial Number: NCI-8200

1. Laboratory of Tumor Pathophysiology
2. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Tumor Cells in Tumor-efferent blood

Previous Serial No.: None

Principal Investigator: T.P. Butler

Other Investigator: P. M. Gullino

Cooperating Units: None

Man Years:

Total: 1 1/2

Professional: 1

Other: 1/2

Project Description:

Objective: Identification of tumor cells in efferent tumor blood with subsequent quantitation of cell shedding by growing, regressing and metastatic tumors.

Methods Employed: Two hormone-dependent (MTW9 and DMBA) and one hormone-independent tumors (Walker 256) have been grown in vivo in rats, using an ovarian pedicle preparation, resulting in an isolated tumor served by a single artery and a single vein. Under anesthesia the efferent vein was cannulated and the entire efferent blood flow from the tumor was collected over time intervals from 5 minutes to 3 hours. Simultaneously, blood samples were drawn from the jugular vein and carotid artery. Total and differential counts of nucleated cells were performed on all samples, including blood from all three types of growing tumors, and from the regressing MTW9.

Antisera to the tumors were induced in New Zealand rabbits by intravenous or subcutaneous injection of single cell suspensions of minced tumor. Rabbit anti-tumor globulin was prepared by ammonium sulfate precipitation and absorption was carried out against normal rat white blood cells. Indirect immunofluorescence employing fluorescein isothiocyanate-labelled caprine anti-rabbit gamma-globulin was used in testing antisera for specificity and titer.

Major Findings:

- 1) TEB and VB had significantly higher "white cell" counts than did AB, with TEB counts highest (although not significantly different from VB). Differential counts among TEB, VB and AB did not differ significantly. No significant differences in these parameters were observed among growing DMBA, MTW9 or Walker 256, tumors.
- 2) Regressing MTW9 tumor-bearing animals displayed the same pattern of results

as observed in growing tumors.

3) Significant hemoconcentration occurred in the tumors as shown by hematocrits significantly higher in TEB than in VB or AB. Hematocrits were consistently TEB>VB>AB.

4) Rats were consistently anemic and red cell morphology showed many fragmented cells.

5) Tumor cells could not be distinguished with certainty from normal nucleated blood cells on the basis of morphological criteria.

6) It has been possible to produce an antiserum in rabbits which, after appropriate absorption, is specific for MTW tumor cells, and which does not cross-react with any normal constituents of rat blood.

Significance for Cancer Research: A quantitation of the number of cells lost by the tumor is necessary for assessing whether 1) metastasizing tumors differ from non-metastasizing tumors with respect to the number of cells entering the efferent blood, 2) immunological tolerance is related to the number of neoplastic cells released in the general circulation, 3) the growth potential of the mammary tumor cells in blood is comparable to that of the tumor mass, 4) any biological difference between metastasizing and non-metastasizing tumors is related to the capacity of neoplastic cells to survive in blood.

Proposed Course of Research: Indirect immunofluorescence will be used to identify and quantitate tumor cells in the efferent blood of growing, regressing, and metastatic rat mammary tumors.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 8200

Title: Tumor cells in tumor-efferent blood

Principal Investigator: T. Butler

Section:

Lab/Branch: Laboratory of Tumor Pathophysiology

Goal: Identification of tumor cells in efferent tumor blood with subsequent quantitation of cell shedding by growing, regressing and metastatic tumors.

Approach: Hormone-dependent rat mammary carcinomas are isolated in rats so that they are served by a single artery and vein. The tumor efferent blood is collected and compared with aortic and venous blood in terms of hematocrit, nucleated cell count and differential nucleated cell count.

Antisera to the tumors are prepared in rabbits and subjected to ammonium sulfate precipitation and absorption with normal rat liver and white blood cells.

Indirect immunofluorescence employing caprine anti-rabbit gamma globulin will be used to detect and quantitate tumor cells in concentrated white cell fractions of the tumor efferent blood of growing, regressing and metastatic rat mammary tumors.

Progress:

- 1) Tumor efferent blood and venous blood from both growing and regressing tumors have been found to contain significantly higher nucleated cell counts than carotid arterial blood, after correction for hemoconcentration within the tumor.
- 2) No significant differences in differential counts could be determined among different samples, and tumor cells could not be distinguished with certainty from normal white blood cells on the basis of morphological criteria.
- 3) Rabbit anti-MTW9 serum has been produced which does not cross-react with normal blood cells, and immunofluorescent quantitation of tumor cells in efferent tumor blood has begun.
- 4) Antisera to W256 and DMBA are presently being purified and should be used for quantitation shortly.

Significance for Cancer Research (NCP Objective 4 Approach 2): A quantitation of the number of cells lost by the tumor is necessary for assessing whether

- 1) Metastasizing tumors differ from non-metastasizing tumors with respect to the number of cells entering the efferent blood,
- 2) Immunological tolerance is related to the number of neoplastic cells released in the general circulation,
- 3) Any biological difference between metastasizing and non-metastasizing tumors is related to the capacity of neoplastic cells to survive in blood.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-8201

1. Laboratory of Tumor Pathophysiology
2. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Mechanisms of Tumor Angiogenesis.

Previous Serial No.: None

Principal Investigator: Dr. Michael A. Gimbrone, Jr.

Other Investigators: Dr. P. M. Gullino

Cooperating Units: None

Man Years:

Total: 1 1/2

Professional: 1

Other: 1/2

Project Description:

Background: The growth of solid tumors depends upon new blood vessel formation in surrounding host tissues. Folkman and co-workers have isolated a tumor cell factor (Tumor-angiogenesis factor, TAF) which causes new blood vessel formation in vivo and may be an important mediator of naturally occurring tumor angiogenesis. The inhibition of this factor conceivably could provide a new approach to the control of solid tumor growth and metastasis.

Objectives: The current project is concerned with the mechanisms of tumor-induced neovascularization and consists of 2 parts:

- 1) The development of in vitro test systems to identify endothelial cell stimulating factors isolated from or released by tumor cells, and to determine their mechanisms of action.
- 2) The application of recently developed model systems (1,2) for studying the neovascular responses to tumor growth in vivo.

Methods:

- 1) Human endothelial cells are isolated from fresh umbilical cord veins by collagenase digestion and cultured in vitro. Confluent monolayer cultures of these target cells in microtitre wells provide a test system for microgram quantities of extracts. Stimulation of DNA-synthesis is assayed by scintillation counting of ³H-thymidine incorporation. The response of control cultures of human and mouse fibroblasts is compared.
- 2) Live tumor cells or tumor cell extracts are implanted into the normally avascular, transparent cornea of the rabbit eye. New blood vessel formation is observed directly with the slitlamp stereomicroscope and quantitated by colloidal carbon injection. Fragments or extracts of non-malignant, pre-malignant and frankly cancerous tissues can thus be compared for their neovascular activity. The anti-angiogenic effect of drugs or anti-sera can be directly accessed in this system.

Major Findings:

- 1) Reliable culture techniques have been developed for human endothelial cells in primary and secondary cultures. The identity of these cells has been established by electron microscopy and fluorescent antibody staining. The parameters for a DNA-synthesis assay have been determined and consistent responses have been obtained to standard test mitogens. Samples of TAF from Dr. Folkman's laboratory are now being tested in preliminary experiments.
- 2) Preliminary experiments with xenografts of rat and mouse tumors have shown that the rabbit cornea is an immune-privileged site for tumor growth and vascularization, thus making it feasible to study human biopsy material in this model.

Significance for Cancer Research:

- 1) Prevention of neovascularization has already been shown to block the growth of a solid tumor at an early stage (1). An in vitro assay for critical mediators would speed their characterization and the search for possible inhibitors.
- 2) The cornea implant system may provide an in vivo assay for malignant potential which could be applied to human biopsy material. It also allows testing possible anti-angiogenesis drugs or other agents.

Proposed Course of Research:

- 1) If cultured endothelial cells are not stimulated by TAF fractions, mixed cell cultures with live tumor cells and endothelium will be studied to ascertain whether direct inter-cellular stimulation in fact occurs. Other types of endothelial cells, e.g., from capillaries rather than large vessels, will also be tested.
- 2) A protocol for the study of mouse mammary lesions, pre-neoplastic and neoplastic, will be started in the near future, to determine whether angiogenesis is a reliable indication of the latent malignant potential of a tissue.

Honors and Awards: None

Publications:

1. Gimbrone, M.A. et al., Tumor Dormancy In Vivo by Prevention of Neovascularization. J.Exp.Med. 136: 261-276, 1972.
2. Gimbrone, M.A. et al., Tumor Angiogenesis: Iris Neovascularization at a Distance from Experimental Intraocular Tumors. J.Nat.Cancer Inst. 50: 219-228, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 8201

Title: Mechanisms of Tumor Angiogenesis

Principal Investigator: Michael A. Gimbrone, Jr. M.D.

Section:

Lab/Branch: Laboratory of Tumor Pathophysiology

Goal: 1. Develop in vitro tissue culture test systems for endothelial cell mitogens isolated from or released by tumor cells.

2. Determine the tumor-specific nature of neovascularization induced in vivo in corneal implant system.

Approach: 1. Development of cell cultures of human and animal endothelial cells, which can be used in radioisotope labeling assays for DNA synthesis. Test ability of tumor factors isolated from tumor cells, or released directly by live tumor cells in tissue culture, to stimulate DNA-synthesis in these endothelial cells. Compare with extracts of normal cells. Endothelial cells appear to represent a "target cell" for action of tumor factors. Folkman, et al. have isolated various tumor factors which have the unique ability to stimulate DNA-synthesis in endothelial cells in vivo. Our approach is to reproduce this phenomenon in vitro in a controlled environment, thus allowing quantitation of activity of tumor factors which has not been possible in existing in vivo test systems.

2. Corneal implants of pre-malignant lesions in the C3H mouse mammary tumor model will be studied for their ability to evoke neovascularization, as a new approach to accessing the malignant potential of atypical tissues.

Progress:

1. Culture techniques have been developed which allow the growth of proved human endothelium in vitro. Parameters for an in vitro assay of DNA-synthesis in these cells have been determined. Positive responses to standard non-specific plant mitogens have been obtained. Preliminary tests of tumor-angiogenesis factors supplied by Dr. Folkman's laboratory are in progress.

2. A variety of normal and malignant tissues which have been implanted into corneal pockets are being studied for vascular responses.

Significance for Cancer Research (NCP Objective 4 Approach 3):

1. Development of an in vitro assay for tumor-angiogenesis factors would greatly speed their isolation and characterization.

2. Demonstration of a consistent relationship between malignant potential of atypical tissues and their ability to evoke a neovascular response in animal test systems would provide a valuable insight into the nature of tumor angiogenesis and perhaps prove useful as a diagnostic tool.

Level of Effort: Professional Man-Years: 1

LABORATORY OF THEORETICAL BIOLOGY

SUMMARY

July 1, 1972 - June 30, 1973

Summary Report

The Laboratory of Theoretical Biology has only been in existence for one year - and this is its first annual report. During this year all the personnel were recruited. Some of the personnel started new projects and some continued with projects they had previously been engaged in. The Laboratory has one Section, Mathematical Biology, headed by M. Berman.

In general, the activities of the Laboratory fall into three areas: theory of biological phenomena, mathematics and computations related to the quantification of biological systems and modeling of kinetic-metabolic systems. The first area covers mostly problems related to membrane phenomena (Blumenthal) and some modeling theory (Berman). The second covers modeling techniques using computers (Berman). The development of general purpose computer programs (Berman, Weiss) computer communications interfacing (Bidinger) and mathematics of numerical analysis (Chu). The third area covers the modeling of specific metabolic (Phair, McGuire, Dubois, Leme, Berman). Much of the latter is done in collaboration with experimental groups at NIH and elsewhere (Iodine - NIAMD; Lipids - NHLI, U. of Florida, U. of Brussels; Glucose-insulin - NICHD; GI - NIMH; Ca - U. of San Paulo).

Theoretical Biology

Most of the work in this area is carried out by Dr. Blumenthal. His interests are concentrated on membrane models from the point of view of transports, structural organization and cooperativity. In connection with this he has been developing theory for membrane transport which may account for or be applied to cellular control processes at the membrane level. The transport proteins in the membrane are assumed to undergo cooperative conformational changes while mediating transport. When the non-linear membrane transport processes are kinetically coupled to enzymatic degradation or lateral diffusion interesting dynamic patterns are exhibited by the models: multiple steady state transitions, overshoot of concentration levels within the cell resembling action potentials in nerve membranes, oscillations, spatial patterns and propagation of chemical waves. These models may be applied to transitions of respiratory states in mitochondria, of uptake of metabolites in transformed cells, of levels of inducer in differentiating cells, to morphogenesis and to aggregation of membrane surface proteins.

Dr. Blumenthal is also involved in the study of physical mechanisms for ion transport in synthetic and ultimately biological membranes. In connection with this he's involved in the development of the lipid bilayer membrane as an assay for ion-conductance inducing materials isolated from natural cell

membranes. This involves experimental work and is done in cooperation with the Biophysics Laboratory, NINDS.

Mathematics and Computations

The SAAM computer program is a general purpose program developed by our group (while at NIAMD) over the past 14 years for the modeling of various biological systems, especially those dealing with kinetics. It embodies, in addition to a general modeling philosophy, most of the computational and mathematical experience we have gained over the years. This program is widely used by our group and by other investigators here at NIH and elsewhere. As the modeling is getting more and more sophisticated, greater demands are put on the SAAM program, its capabilities, ease of handling and computational efficiency. Because of this there is a continuous effort to expand the SAAM capabilities and to improve the mathematical and computational techniques in it.

Dr. Chu is the mathematician in residence concerned with the numerical analysis procedures contained in the program. This includes the rigorous testing of existing procedures and the development of new, more efficient methods. For the modeling of large systems (an ever increasing need) considerably greater solution efficiencies are required and new techniques have to be introduced. There is also a problem of precision which can seriously deteriorate under certain solution conditions. Dr. Chu has been able to develop much improved precision for the solution of a class of differential equations at no serious increase in solution time. This will be most helpful in dealing with larger systems. Efforts to extend this to broader classes of problems is in progress.

The programming, updating, and restructuring of SAAM is done by M. Weiss. The SAAM program is huge (about 30,000 FORTRAN statements in about 200 subroutines), and it takes considerable care and knowledge of many details to perturb it without adverse repercussions. The implementation of new features in SAAM are thus very delicate operations requiring comprehensive understanding and careful testing. This year a major addition to SAAM was made in the form of a new computational element called a 'delay'. This, in effect, permits the solution of differential - difference equations - important in systems containing delays. Considerable restructuring of SAAM was necessary to introduce this element and to allow for future extensions of its power.

A conversational version of SAAM was introduced last year (Berman) and is still being developed. Computer access seems to be a major obstacle to its routine use.

Modeling

Several systems are actively being modelled by this group, usually in collaboration with experimental groups at NIH and elsewhere. Part of the reason for modeling these systems is the fact that the modeling process itself is becoming quite sophisticated and requires considerable background and

experience. The experimental groups usually do not have the personnel with the background. Furthermore, the modeling has grown to a point where information from two or more experimental groups have to be pooled - resulting in large modeling systems. These factors combined - the sophisticated modeling, pooling of information and knowledge of the physiology - create a unique requirement which can probably best be met by a theoretical group like ours. The dual role of the group in developing methodology and in applying it to real problems result in strong mutual support and reinforcement.

Another objective of our modeling is to establish reasonable quantitative model bases that may be used to design new experiments and to elucidate differences between normal and abnormal physiological states.

The kinetics of iodine and thyroid hormone metabolism has been of interest to us for some time (Berman). In the past year or so, in collaboration with the endocrine group at NIAMD the effects of lithium on the kinetics of iodine were investigated and two sites of action of lithium were identified: the hydrolysis of thyroglobulin in the thyroid and degradation of T_4 . It was also observed that the recovery after lithium is stopped takes considerable time. This prompted the investigation of the kinetics of lithium and its mode of action on hormone degradation (Berman, McGuire).

In collaboration with Dr. M. Hays of the VA Hospital in Buffalo the kinetics of pertechnetate are also being modelled (Berman). Certain similarities in the kinetics of Tc and iodide prompted the modeling of this system.

In collaboration with the New York Hospital, N.Y. (Drs. Belshaw, Barandes, Becker) a model for iodine kinetics in the dog has been developed. Comparisons between it and the model for man have been made with respect to thyroidal handling of iodine and the metabolism of T_4 and T_3 .

The lipoprotein studies have evolved into what we now consider to be a large model. It encompasses the triglycerides, apo-proteins (apo-B and apo-C), of the very low density (VLDL), low density (LDL) and high density (HDL) lipoproteins, their catabolism and relations to precursors involved in their synthesis: free fatty acids (FFA), glycerol, glucose and amino acids. The experimental work is being carried out by 3 groups, one at NIH (Drs. Bilheimer, Levy - NHLI) one at U. of Florida (Dr. Fisher) and one at the U. of Brussels (Drs. Malmendier, Delcroix). The integration of the various findings into a comprehensive model is carried out in our group (Phair, Berman). A most unique theoretical aspect of this project is the fact that these studies complement each other to the point where new features in the overall model are generated that could not have evolved from any one study independently. The comprehensive model evolved includes the oxidative pathways of FFA, glycerol and glucose and their conversion to lipoproteins of various densities, and the delipidation and deproteinization of the lipoproteins - in normal and hyperlipemic populations.

In collaboration with the gerontology Research Center of the NICHD (Drs. Andres, Sherwood, Insel, Kramer, Tobin) we have been studying the kinetics of glucose and insulin, the control of insulin on glucose metabolism and the

control of glucose on insulin secretion in man, under normal and abnormal conditions. A model for the insulin and glucose subsystems has been developed and control sites and modes of control of insulin on glucose have been identified. A model for the control of glucose on the secretion of insulin is presently being tested (Berman).

Models for calcium and magnesium metabolism have also been developed in the past (Berman). Further, work on the calcium model is presently being carried out (Leme) on a Brazilian population. Although the data fit our previously proposed models certain anomalies related to the specific activities in urine and early urinary excretions remain unresolved. Magnesium kinetics in hyperparathyroid patients are also being investigated in collaboration with Dr. Avioli (Washington U., St. Louis). This is an outgrowth of earlier work on Mg kinetics carried out with Dr. Avioli. (Leme, Berman).

A model for the kinetics of methotrexate in patients with carcinoma was developed (Leme, Berman) in collaboration with Dr. Creaven of the VA Hospital, D.C. The model suggests that some differences exist in the handling of the drug between large and small doses, and that delivery of the drug to its site of action may not be linear. A comparison with models developed for methotrexate kinetics in rats has also been made.

Regulation of gastric emptying and motility under normal conditions and after surgery and the administration of drugs has been studied in the dog (Dubois, Berman). A model incorporating the effect of stomach acidity on its rate of emptying and the control of acid secretion has been developed. This model shows mechanisms of action that can be interpreted well in terms of actions by the sympathetic nervous system.

Serial No. NCI-8300

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: SAAM Computer System

Previous Serial Number: None

Principal Investigator: Dr. Mones Berman

Other Investigators: Marjory F. Weiss

Man Years:

Total:	1.3
Professional:	1.3
Others:	0

Project Description:

Objectives: To develop a general purpose computer program for modeling biokinetic systems that may readily be used by investigators not sophisticated in mathematics or programming. Initiated in 1959, the program continues to be expanded and revised as new features are added.

Major Findings: A major revision of the structure of the program was undertaken and completed to permit additional features to be added to the program. Greater efficiency in operation and greater ease of use were additional results of this revision. New features have permitted the solution of some problems not previously within the scope of the SAAM program.

A revision of the SAAM Manual has been partially completed and will describe the newest version of the program.

Broadening of the scope of the program, its use as a conversational tool and refinement of mathematical and statistical methods will continue.

Serial No. NCI-8301

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Analysis of Kinetic Data and Modeling

Previous Serial Number: None

Principal Investigator: Dr. Mones Berman

Other Investigators: Dr. Robert McGuire, Lab. Theoretical Biol., NCI
Dr. M Hays, VA Hospital Buffalo, N.Y.
Dr. J. Robbins, NIAMDD
Dr. R. Andres, NICHD
Dr. C. Kramer, NICHD

Man Years:

Total:	2.0
Professional:	2.0
Other:	0

Project Description:

Objectives: Analysis of data on metabolic systems, and the further development of modeling techniques through the use of the SAAM computer program. Major emphasis went into the iodine system and the glucose-insulin control system.

Major Findings: In modeling the effects of lithium in iodine kinetics a new site of action was discovered. In addition to control of the hydrolysis of thyroglobulin, lithium also reduces the peripheral degradation rate of thyroxine. Details of this mechanism are now being investigated.

The kinetics of lithium are also being studied in order to relate its temporal behavior with the onset of the observed effects on iodine. (McGuire).

Studies on the kinetics of pertechnetate are being carried out in collaboration with Dr. M. Hays of the VA Hospital in Buffalo, Tc behaves in many ways like iodine. A model has been proposed and sites of competition with iodine have been identified.

A model for the glucose-insulin system has been developed over the past few years in collaboration with the Gerontology Research Center, NICHD, Baltimore.

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1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

First, the insulin and glucose subsystems were defined. The control of glucose utilization by insulin has also been characterized. A model for the secretion of insulin from the pancreas and its control by glucose is now being tested.

Publications:

Berman, M.: Iodine Kinetics. In Methods of Investigative and Diagnostic Endocrinology. North-Holland Publishing Co., Amsterdam, Holland, 1972, pp. 172-203.

Temple, R., Berman, M., Robbins, J., and Wolff, J.: The Use of Lithium in the Treatment of Thyrotoxicosis. J. Clin. Invest., 51: 2746-2756, 1972.

Temple, R., Berman, M., Carlson, H., Robbins, J., and Wolff, J.: The Use of Lithium in Graves' Disease. Mayo Clin. Proc. 47: 872-878, 1972.

Serial No. NCI-8302

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Computational Mathematics

Previous Serial Number: None

Principal Investigator: Dr. Sherwood C. Chu

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: To evaluate, develop and implement mathematical techniques used in the modeling and simulation of biological systems. In particular, to develop an efficient method for the numerical solution of systems of ordinary differential equations.

Methods Employed: The primary mathematical tools needed to evaluate or develop an algorithm are from the theories of differential equations, numerical analysis, and linear algebra. Computer programming provides the means for implementation.

Major Findings: A numerical procedure for the solution of a class of initial value problems - those corresponding to linear systems with constant coefficients - has been implemented. It is more accurate, although more time consuming, than a similar method now in use.

An improvement is made on one of the solution routines in the SAAM program.

Significance to Biomedical Research: Sound computational techniques facilitate and permit the mathematical analysis of complex biological systems.

Proposed Course: The analysis of the various mathematical aspects of biological modeling will be continued. An effort will be made to broaden the applicability of existing algorithms to more general systems as well as making them more computationally efficient.

Serial No. NCI-8303

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Ion Transport Through Modified Lipid Bilayer Membranes

Previous Serial Number: None

Principal Investigator: Dr. Robert Blumenthal

Cooperating Units: Laboratory of Biophysics, NINDS, NIH
Laboratory of Neurochemistry, NINDS, NIH

Man Years:

Total:	.5
Professional:	.5
Others:	0

Project Description:

Objectives: To study the physical mechanisms for ion transport in synthetic membranes. To develop the lipid bilayer membrane as an assay for ion-conductance inducing materials isolated from natural cell membranes. The ultimate goal of the synthetic membrane studies is the elucidation of the molecular mechanism of membrane excitation and active transport.

Methods Employed: The bilayer membranes are formed from natural membrane extracts, oxidized cholesterol or pure lipids in an aperture between two electrolyte solutions. The electrical properties of the membranes are measured before and after application of an activating factor, such as EIM (a bacterial protein which induces excitability). Measurement of electrical fluctuations provide information about the molecular rearrangements of EIM in the membrane. The study of active transport involves similar measurements on a sodium dependent ionophore isolated by Drs. A. Shamoo and W. Albers, Laboratory of Neurochemistry, NINDS from a tryptic digest of the $\text{Na}^+\text{-K}^+$ ATPase active transport system of electric eel and kidney.

Major Findings: The kinetics of EIM-induced conductance changes were studied by the method of analysis of discrete conductance fluctuations. The EIM conducting channels were shown to obey Poisson statistics with transition rates which vary in a simple exponential manner with the transmembrane electrical potential. The measurements of microscopic conductance fluctuations were shown to be completely consistent with the macroscopic parameters

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1. Laboratory of Theoretical Biology
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determined from voltage clamp experiments. This complete specification of the kinetic parameters of a system of ion conducting channels of molecular dimensions has allowed us to determine the nature of the energy barriers involved in the activation of the excitable units.

Significance to Biomedical Research and the Program of the Institute: The voltage dependent permeability properties of the EIM doped bilayer bears many similarities to those of natural excitable membranes. The analysis of single conducting channels has given support to the idea of discrete conducting channels in excitable membrane. With respect to cancer, possible regulatory functions of action potentials and ion gating include cell division, parthenogenic activation of ova, pseudopod formation and gating of cell junctions. The bilayer has proven to be a sensitive probe for the molecular properties of ion conducting channels in membranes. The extension of the bilayer techniques as an assay for the sodium dependent ionophore of the $\text{Na}^+ - \text{K}^+$ ATPase offers the promise of the reconstitution of the active transport process. Ion pumping is of particular importance in the control of cellular functions.

Proposed Course of the Project: Experiments on the ATPase ionophore are in progress to determine the ionic specificity of the active fraction and whether the ionophore acts as a carrier or channel within the membrane.

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1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Dynamic Membrane Transport Models

Previous Serial Number: None

Principal Investigator: Dr. Robert Blumenthal

Man Years:

Total:	.5
Professional:	.5
Others:	0

Project Description:

Objectives: To develop models describing transformations of levels of cellular activity induced by transient environmental stimuli at the membrane level.

Methods Employed: Expressions are derived for the transient and steady state concentration levels of metabolite on the membrane surface. Simulations are carried out making use of the PDP-10 of the central NIH computer facility.

Major Findings: In the models membrane transport proteins are assumed to undergo cooperative conformational changes, while mediating transport. When the non-linear membrane transport process is kinetically coupled to enzymatic degradation or lateral diffusion over the membrane surface the system will exhibit interesting dynamically patterned behaviour: Multiple steady state transitions, overshoots of the concentration level within the cell resembling action potentials in nerve membranes, oscillations, spatial patterns on the membrane surface and propagation of chemical waves.

Significance to Biomedical Research and the Program of the Institute: The significance may be listed in terms of the major findings of the formal models: 1) Biological examples of multiple steady states include transitions of respiratory states in mitochondria, of uptake of metabolites in transformed cells and of levels of inducer in differentiating cells. 2) Sustained oscillatory behaviour of cells may account for a time basis for development e.g. slime mold amoeba send out periodic waves of cyclic AMP upon aggregation and morphogenesis. 3) Pattern formation of molecules on the membrane surface may account for the dynamic architecture of membranes. The models may specifically be applied to transformations of surface antigen arrangements on the lymphocyte membrane.

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This project most closely supports Objective 4, Approach 5 of the "National Cancer Plan" by developing mathematical models of the cancerous process.

Proposed Course of Study: The models will be further developed in relation to biological data on dynamic membrane phenomena.

Honors and Awards: Invited to lecture at the Aharon Katchalsky Memorial Symposium, Berkeley, California.

Publications:

Blumenthal, R. P.: Dynamic Patterns in Active Transport. Israel J. Chem., (1973) in press.

Katchalsky, A., Rowland, V., and Blumenthal R. P.: Dynamic Patterns in Brain Cell Assemblies. Neurosciences Res. Prog. Bull., (1973) in press.

Serial No. NCI-8305

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Remote Communications Computer System

Previous Serial Number: None

Principal Investigator: Peter A. Bidinger

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: To develop a remote terminal computer operating system which will interface with various other computers and utilize various on line input-output devices.

Major Findings: A system has been developed using a PDP-11 which communicates with Univac 1108's and Univac 418's both on 2400 baud and 4800 baud telephone lines. An emulator has been developed to act as an Univac 1004. Documentation is also being written to provide investigators not sophisticated in computer operation with a convenient access to the system.

Serial No. NCI-8306

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Kinetic Modeling of Human Plasma Lipoprotein Metabolism

Principal Investigators: Robert Phair and Mones Berman

Other Investigators: Waldo R. Fisher (U. of Florida, Gainesville)
David W. Bilheimer (NHLI)
Robert I. Levy (NHLI)

Cooperating Units: University of Florida, Gainesville

Section on Lipoproteins
Molecular Disease Branch
National Heart and Lung Institute

Man Years:

Total:	1.2
Professional:	1.2
Others:	0

Project Description:

Objectives: We hope to reach a quantitative understanding of human plasma lipoprotein metabolism in health and disease. Integration of our experimental data and conclusions with previous work in this laboratory and with results obtained by other investigators is a major objective.

Methods: Mathematical modeling is our major tool. Broadly, this includes the application of quantitative physical techniques to both the analysis of experimental data and the integration of conclusions. Formal models allow the rigorous testing of hypotheses and are used in the design of subsequent experiments.

Major Findings: Continuing analysis of data obtained using ^3H -leucine as a tracer for the lipoprotein apoproteins has led us to think of very low density lipoproteins (VLDL) as a succession of progressively more dense particles all capable of generating low density lipoproteins (LDL). This is in contrast to the widely held view that the most dense VLDL particle gives rise to the least dense LDL particle as a consequence of lipid removal.

Investigation of the same system using exogenous ¹²⁵I labeling of the apoprotein tyrosine residues has revealed some metabolic differences between normals and hyperlipoproteinemic patients. In general, the rate of VLDL to LDL conversion, in these patients, is reduced by approximately a factor of three compared to normals. In types I, II and IV hyperlipoproteinemia a delay in this conversion pathway is required to explain the experimental data. In type III hyperlipoproteinemia this delay is not required but analysis of kinetic data from patients with this disease has focused our attention on the role of the β -migrating VLDL species known to form in type III individuals. It is possible that this species does not act as a precursor for LDL.

Significance: Understanding of normal plasma lipoprotein metabolism and identification of the metabolic defects in the hyperlipoproteinemias is important both for the treatment of these patients and for the insight gained into the general problem of atherosclerotic vascular disease.

Proposed Course: Study of plasma lipoprotein metabolism from many points of view is a long-term project in this Laboratory. We intend to continue formal integration of our results with those of other workers and to "feed back" on the design of future experiments to be carried out in the laboratories of our collaborators.

Awards: Both of the principal investigators were awarded small grants by the AMP Bioresearch Foundation, San Francisco, CA to defray expenses incurred by attending the 1973 Deuel Conference on Lipids.

Serial No. NCI-8307

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Modeling of Gastric Secretion and Emptying Regulatory Processes

Previous Serial Number: None

Principal Investigator: Dr. Andre T. Dubois

Other Investigators: Dr. M. Berman

Man Years:

Total:	.7
Professional:	.7
Others:	0

Project Description:

Objective: Gastric emptying was previously studied in dogs (Amer. J. Dig. Dis., 1973). To examine the intricate secretory and emptying processes occurring during the digestion of a liquid meal, a mathematical model using the SAAM25 program based on well established regulatory mechanisms was constructed. It was able to predict the measurements previously recorded (Gastroenterology, April 1973). To further determine the adequacy of the model, new experiments were performed.

Methods: An esophagostomy was realized in 4 dogs as previously described (Amer. J. Dig. Dis., 1973). After a recovery period of 3 weeks, the concentrations of acid, Na^+ , K^+ and Cl in the gastric contents were measured in the basal state and during the digestion of a Phenol red tagged test meal. The experiments were repeated with an acid test meal (50 mEq/l) and during the stimulation of gastric secretion with a continuous infusion of pentagastrin (6 mg/kg/h). The model was adapted in order to predict the measurements recorded during this new series of experiments.

Major Findings: The acid meal slows down gastric secretion and emptying. Pentagastrin has a slowing effect on gastric emptying for which the increased acid concentration in the gastric contents does not account. The same measurements are going to be performed in normal volunteers and various pathological situations.

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1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Significance: This method of kinetic analysis provides ground for a better understanding of the regulation processes in the digestive tract.

Publication:

Dubois, A., Watanabe, A. M., and Kopin, I. J.: Postoperative Gastric Ileus.
Am. J. of Digestive Diseases, 18: 1, 1973, 39-42.

Serial No. NCI-8308

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Study of Gastrointestinal Propulsion in the Rat; Role of Noradrenergic Nerves in the Postoperative Ileus

Previous Serial Number: None

Principal Investigator: Dr. Andre T. Dubois

Cooperating Units: Laboratory of Clinical Science, NIMH

Man Years:

Total:	.5
Professional:	.3
Others:	.2

Project Description:

I. Role of Noradrenergic Nerves

Objective: In humans, gastrointestinal propulsion is slowed down during the first few days after abdominal operations. A similar situation has been produced in the rat 12 hours after a test surgical operation. In this animal, chemical sympathectomy prevents this postoperative ileus. To examine if this postoperative depression of gastrointestinal motility is related to sympathetic hyperactivity, the turnover of norepinephrine in the digestive tract has been evaluated in rats.

Methods: The adrenergic nerve terminals are able to take up tyrosine, to synthesize norepinephrine and to release it. This release, and consequently the rate of synthesis are influenced by nerve impulses. Sympathetic activity can thus be evaluated either by measuring the rate of synthesis of norepinephrine from C¹⁴ tyrosine or the decline of norepinephrine after blockade of synthesis.

Major Findings: Both the rate of synthesis and of decline are increased during the occurrence of postoperative ileus, which indicates that the sympathetic nervous system is activated in this situation.

Serial No. NCI- 8308

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

II. Role of the Adrenal Glands

Objectives: The adrenal glands are stimulated by a surgical stress; to determine the influence of this factor on gastrointestinal propulsion, unoperated and operated rats were studied; the effect of previous adrenal demedullation on gut motility was examined.

Methods: Gastrointestinal propulsion was determined by measuring the propulsion of a C^{51} tagged test meal, (Ann. Surg., 1973, in press). Demedullation was surgically realized 2 weeks prior to the study of gastrointestinal propulsion.

Major Findings: No difference was observed between gastrointestinal propulsion of sham demedullated and demedullated rats. Both groups displayed the same postoperative depression of gastrointestinal motility. In the rats in which the adrenal medulla has been removed surgically, plasma catecholamines are dramatically reduced. Plasma corticosterone, which normally rises after surgery, remains unchanged in demedullated rats.

Significance: These observations indicate that the adrenal gland plays only a minor role, if any, in the postoperative ileus.

Publication:

Dubois, A., Weise, V., and Kopin, I. J., Postoperative Ileus in the Rat: Physiopathology Etiology and Treatment. Annals of Surgery, 1973 (In press).

Serial No. NCI-8309

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Kinetics of Methotrexate in Patients with Carcinoma

Previous Serial Number: None

Principal Investigator: Paulo R. Leme

Other Investigators: Dr. Patrick J. Creaven
Dr. Larry M. Allan
Dr. Mones Berman

Cooperating Units: Oncology Service, Veteran Administration Hospital
Washington, D.C.

Man Years:

Total:	1.3
Professional:	1.0
Others:	0.3

Project Description:

Objectives: Establish a model for methotrexate kinetics in patients with advanced cancer and to evaluate variations in treatment schedules.

Methods: After a single i.v. injection of [3', 5', ³H] methotrexate, plasma and urine radioactivity was determined. Analysis of data were performed in terms of a compartmental model using a digital computer and the program SAAM. Parameter values were established and their changes as a function of MTX dose level investigated.

Major Findings: According to the model, plasma MTX consists of at least two components, only one of them serving as a precursor for the urinary MTX. This moiety, has a volume of distribution comparable to extracellular space, and exchanges with another compartment, which reflects partially the intracellular space. The other plasma moiety is probably a contaminant or a by product metabolite.

Urine data suggest a delay (up to 6 hours) in the excretion path. Urine did not account for all the MTX injected and a 2nd loss path had to be introduced.

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1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Significant changes attributable to large MTX doses were a two fold decrease in the rate of urinary excretion and a comparable increase in the 2nd loss pathway. Another major change is an increase by a factor of 1.5 in the availability of the drug in the 'extracellular' compartment, as measured by the time integral of the plasma curve.

Significance to Biomedical Research: The prediction of spatial and temporal distribution of MTX as derived through the model is a relevant aspect to the understanding and design of cancer chemotherapy.

Serial No. NCI-8310

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Compartmental Analysis of Calcium Metabolism in Man

Previous Serial Number: None

Principal Investigator: Paulo R. Leme

Other Investigators: Dr. Bernardo L. Wajchemberg
Dr. Mones Berman
Dr. Moacir Novaes

Cooperating Units: Fundação de Amparo A Pesquisa do
Estado de São Paulo, Brazil

Man Years:

Total:	2.0
Professional:	1.5
Others:	.5

Project Description:

Objectives: To determine the parameters of a previously established model of calcium metabolism in a normal Brazilian population.

Methods: Ten normal young men were injected intravenously with a single dose of $^{47}\text{CaCl}_2$. Serum, urinary, fecal and whole-body radioactivity was measured from 1 minute to 50 days and the results analyzed jointly with data obtained from a simultaneous metabolic balance study, using digital computer techniques.

Major Findings: The data were satisfied by a four compartment model previously established for Ca kinetics. No significant changes were observed in the calculated parameter values, although a lower urinary specific activity than plasma was found. This rather uncommon observation implies that in the plasma there must be at least two different forms of calcium between which the added radiocalcium is not exchangeable, and which are excreted at different rates. Whole-body counting data have shown a significant tracer loss other than urine and feces, which may be attributable to sweat.

Serial No. NCI-8310

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

Significance to Biomedical Research: Further understanding of calcium homeostasis and skeletal metabolism may be obtained by studying the effects of vitamin D, parathormone, thyrocalcitonin, etc., on calcium kinetics.

Serial No. NCI-8311

1. Laboratory of Theoretical Biology
2. Mathematical Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Role of Parathyroid Hormone in Mg Metabolism: Compartment Analysis

Previous Serial Number: None

Principal Investigator: Paulo R. Leme

Other Investigators: Dr. Louis V. Avioli
Dr. Mones Berman

Man Years:

Total: 0.3
Professional: 0.3
Other: 0.

Project Description:

Objectives: To determine changes in Mg metabolism parameters produced by administration of parathyroid extract (PTX) in normals and in patients with hypoparathyroidism and hyperparathyroidism.

Methods: Paired studies before and during PTX treatment were performed in two normal controls, whereas the other 5 patients were analyzed only during PTX administration. After a single i.v. injection of $^{28}\text{MgCl}$, plasma, urine and feces samples were collected from 15 min. to 6 days and assayed for radioactivity.

The data are analyzed by use of a compartmental model previously established for the Mg kinetics. The analysis is carried-out by means of the digital computer program SAAM.

Major Findings: Analysis of this system is still in progress.

Significance to Biomedical Research: Parathyroid hormone is important in the homeostasis of Ca and Mg and the present study is an effort to quantify this control.

SUMMARY OUTLINE
Annual Report of the Immunology Branch, National Cancer Institute
July 1, 1972 through June 30, 1973

INTRODUCTION

I. STRUCTURE AND FUNCTION OF IMMUNE CELL AND NEOPLASTIC CELL MEMBRANES AND CELL PRODUCTS

- A. Membrane Structure
- B. Histocompatibility Antigens
- C. Differentiation Antigens
- D. Receptors
- E. Tumor Cell Antigens
- F. Amyloid

II. CELLULAR IMMUNITY

- A. Suppressor Cells
- B. In Vitro Immunization
- C. Lymphocyte Dependent Antibody
- D. Separation of Lymphoid Cell Populations
- E. In Vitro Methodology

III. GENETICS OF THE IMMUNE RESPONSE

- A. Genetic Control of Response to Staphylococcal Nuclease
- B. Synthetic Polypeptide Antigens
- C. Immunoglobulin Messenger RNA

IV. TRANSPLANTATION IMMUNOLOGY

- A. HL-A and Disease Associations
- B. Graft Versus Host Disease
- C. Antibodies to Histocompatibility Receptors
- D. Animal Transplantation Antigens

V. IMMUNODIAGNOSIS

- A. Carcinofoetal Proteins

VI. IMMUNOTHERAPY

- A. Animal Studies
- B. Leukemia
- C. Basal Cell Carcinoma
- D. Glioblastoma
- E. Melanoma

SUMMARY

Annual Report of the Immunology Branch, National Cancer Institute
July 1, 1972 through June 30, 1973

INTRODUCTION

The Immunology Branch carries out basic immunobiology laboratory investigations with particular emphasis given to studies of the structure and function of cell membranes, cellular immunity, genetics of the immune response and transplantation. The fundamental information obtained is then applied to investigations of the interactions between the immune system and neoplastic cells, as well as to the possible diagnosis and therapy of malignant disease in animal models and in man.

I. STRUCTURE AND FUNCTION OF IMMUNE CELL AND NEOPLASTIC CELL MEMBRANES AND CELL PRODUCTS

A. Membrane Structure. Appreciation of any derangement of the surfaces of malignant cells will depend upon an understanding of normal cell surfaces. Little is known about the structure of cell membranes, although membrane models have been constructed on the basis of thermodynamic considerations. A major protein constituent of red cell membranes has been isolated and almost totally sequenced (5019). The results provide the first structural evidence supporting a lipid bilayer model with intercalated proteins containing a hydrophobic portion buried in the lipid bilayer and a hydrophilic portion exposed to the cell surface.

B. Histocompatibility Antigens. Histocompatibility antigens are normal cell surface constituents of unknown structure. Their great importance as recognition signals for cell-cell interaction, as well as their possible role in the genetic control of immune responsiveness makes their structural analysis of great importance. Methods have been devised to solubilize HL-A containing membranes in a state such that antigenic activity is retained (5006). The HL-A antigen density fluctuates during the cell cycle with increased density in S and G₂ phases (5017). Treatment of lymphoid cell surfaces with neuraminidase reveals an antigen reactive with "natural" antibody present in normal human serum (5017), and this may have implications for the mechanism whereby neuraminidase treatment increases the immunogenicity of cells.

C. Differentiation Antigens. Differentiation antigens provide markers important in the recognition and separation of populations of immunocompetent cells. It has been possible to develop heterologous antisera that differentiate human lymphoid cells of thymic (T) and nonthymic (B) origins (5030, 5031).

D. Receptors. Receptors provide additional markers useful in recognition and separation of populations of cells, as well as in functional studies. Receptors for aggregated human IgG have been identified and characterized and

shown to be reliable markers for B cells (5019). Unexplained high numbers of cells with surface IgD have been observed in normal peripheral blood (5002). Receptors for mitogens (5018), insulin and growth hormone (5004) have been detected and isolated, and attempts are being made to purify them.

E. Tumor Cell Antigens. Tumor cell antigens useful for possible immunotherapeutic approaches presumably will have to be cell surface membrane antigens. Antigenic analysis of blast cells from acute lymphocytic leukemia has revealed an apparently leukemia specific cell surface antigen (5006). A similar antigen has been found on the surface of human, but not murine, cells after they have been infected with murine leukemia virus. The implications of these observations for the etiology and therapy of leukemia are being pursued. Attempts are being made to raise antisera against human tumor cells (5029), particularly against CLL cells where parental cells can be used for cross absorption (5024) and in multiple myeloma, where the idiotypic specificity of the immunoglobulin may serve as a tumor specific antigen (5022).

F. Amyloid. The identification of immunoglobulin as the major protein component of some human amyloid has been strengthened by additional observations. In addition, a new form of amyloid has been identified and the major protein sequenced (5002).

II. CELLULAR IMMUNITY

The role of various lymphocyte populations in the recognition of tumor cells, as well as in effector cell activity against tumor cells, is under investigation.

A. Suppressor Cells. In vitro studies have provided further support for the existence of lymphoid cells that "suppress" cell mediated immunity and indicate that the thymus of adult animals is the source of these suppressor cells (5003). In vivo studies are in progress to determine the role of suppressor cells in the tumor bearing animal.

B. In Vitro Immunization. One possible reason that tumors grow in the face of an immune response directed against them is that the immune response is quantitatively inadequate. In vitro techniques for immunizing lymphoid cells might be very useful, and accordingly, methods have been set up for immunizing human lymphoid cells in vitro (5016).

C. Lymphocyte Dependent Antibody. A mechanism has been demonstrated whereby antibody can coat target cells and thereby sensitize them for destruction by nonimmune lymphoid cells. It has been shown that the antibody is an IgG, and that the attacking lymphoid cell is probably a B cell, or a subpopulation of B cells (5016).

D. Separation of Lymphoid Cell Populations. Attempts have been made

to separate immunocompetent cells into functionally different subpopulations by various techniques. Cell mediated cytotoxicity has been shown to be a function of lymphoid cells with histamine receptors on their cell surfaces, while precursors to these cytotoxic cells lacked such receptors (5028). Additional receptors are being studied to determine the significance of these findings.

E. In Vitro Methodology. Micromethods for studying cellular immunity in vitro have been developed and applied to investigations of interactions between the immune system and tumor cells, as well as to the nature of the cellular immune response (5032, 5014). Further studies of the assay used by the Hellstroms has demonstrated that "innocent bystander" killing occurs and establishes a difference between this assay and the 4-6 hour cell mediated cytotoxicity assay, in which complete specificity is demonstrated.

III. GENETICS OF THE IMMUNE RESPONSE

Previous work has indicated that immune reactivity to defined antigens is under genetic control and that the genes appear to be linked to histocompatibility genes.

A. Genetic Control of Response to Staphylococcal Nuclease. These observations have been extended to the more complex antigen staphylococcal nuclease and both mice and rats have been shown to demonstrate genetic control for the synthesis of antibody to this antigen. In addition, it has been possible to demonstrate in mice that cell mediated immunity is also under genetic control, making it possible to study the structural relationships between the cell surface antigen receptors and H-2 antigens in congenic mice (5025, 5026).

B. Synthetic Polypeptide Antigens. Studies with synthetic polypeptide antigens that are under genetic control are being pursued to determine whether some immune response genes are linked to genetic markers other than H-2 (5027).

C. Immunoglobulin Messenger RNA. Considerable theoretical speculation has gone on concerning the number of activated immunoglobulin structural genes in somatic as opposed to germ line cells. Determination of the number of genes could be approached, by hybridization experiments if one had purified immunoglobulin messenger RNA. Messenger RNA from mouse myeloma cells has been isolated and purified and used in a cell free biosynthetic system. For the first time, it has been possible to directly prove that the apparent immunoglobulin mRNA can be translated into the appropriate product. Amino acid sequence studies are consistent with this translation, although it appears that there is an extra bit of polypeptide chain of some 20 amino acid length preceding the initiation of the light chain sequence (5002). Hybridization studies will be performed.

IV. TRANSPLANTATION IMMUNOLOGY

A. HL-A and Disease Associations. Associations have been sought between human HL-A antigens and certain diseases. A striking association has been demonstrated between HL-A2 and length of survival in patients with acute lymphocytic leukemia. Additional relationships between incidence of certain diseases and HL-A type have been demonstrated (5017).

B. Graft Versus Host Disease. Studies directed toward minimizing or avoiding graft versus host disease following transplantation of immunocompetent cells have been carried out in subhuman primates (5011). Possible use of this approach in the therapy of malignancy will depend upon development of methods to avoid GVH.

C. Antibodies to Histocompatibility Receptors. Attempts are being made to develop antibodies of restricted heterogeneity to transplantation antigens and then to develop antiidiotype antibodies against them so that antireceptor specificity may be obtained. If such reagents can be obtained, they will provide potential for modifying the immune response (5021).

D. Animal Transplantation Antigens. A large animal model for studying transplantation antigens has been established in swine. Once bred to an adequate level of homozygosity, and after adequate reagents have been prepared, cells from these animals will be used to provide histocompatibility antigens for structural studies (5023).

V. IMMUNODIAGNOSIS

A. Carcinofetal Proteins. Carcinofetal proteins have been the most likely candidates to date for substances useful in the early diagnosis of cancer. Because of this, structural studies of α -fetoprotein (5019) and carcinoembryonic antigen of colon (CEA) (5018) have been pursued. In addition, a test facility has been established by contract where immunodiagnostic tests can be performed and where samples can be simultaneously tested for CEA, α -fetoprotein and chorionic gonadotrophins (see Contract NO1-CB-33875).

VI. IMMUNOTHERAPY

A. Animal Studies. Immunotherapeutic studies have been carried out in mice using BCG and "deblocking sera." Present studies are directed toward neuraminidase modified cells and their role in the therapy of established tumors (5020).

B. Leukemia. Patients with leukemia are being treated by immunization with allogeneic cultured lymphoid cells and BCG (5006) in a collaborative study with the Hematology and Supportive Care Branch, NCI.

C. Basal Cell Carcinoma. Patients with basal cell carcinoma are being treated with topical dinitrochlorobenzene (DNCB) as an immunotherapeutic approach in collaboration with the Dermatology Branch, NCI.

D. Glioblastoma. Patients with glioblastoma will be sensitized with BCG and treated with PPD intracranially into plastic reservoirs inserted at the time of surgery. This immunotherapeutic project will be carried out in collaboration with the Surgical Neurology Branch, NINDS.

E. Melanoma. Patients with stage II and stage III melanoma will be immunized with neuraminidase treated allogeneic cultured melanoma cells in a randomized study that will be carried out in collaboration with the Medicine and Surgery Branches, NCI.

A major commitment in the field of clinical immunotherapy will be made in the coming year.

Serial No. NCI-5002 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Genetic, Structural and Functional Characteristics of Immunoglobulins and Related Proteins

Previous Serial Number: Same

Principal Investigator: William D. Terry, M. D.

Other Investigators: Israel Schechter, M. D., Ph. D.

Cooperating Units: Dr. Thomas A. Waldmann, Metabolism Branch, NCI, Dr. Ira Green, Laboratory of Immunology, NIAID
Dr. George G. Glenner, Laboratory of Experimental Pathology
NIAMDD

Man Years:

Total: 3 1/8
Professional: 1 1/8
Others: 2

Project Description:

Objectives: The primary objectives of this project are to study the genetics and functional characteristics of human immunoglobulins and relate these characteristics to molecular structure. Finding previously unrecognized forms or categories of immunoglobulin molecules is an important part of this work. As different forms of immunoglobulin molecules are recognized, attempts are made to detect genetic factors in order that the genetic map of structural cistrons coding for immunoglobulin polypeptide chains can be more fully developed. In addition, aspects of the chemical heterogeneity of immune globulins are being studied to improve our understanding of the mechanisms generating the heterogeneity and to determine the possible biologic significance of this heterogeneity. The different forms of immunoglobulin molecules continue to be studied for correlations between molecular structure and biologic functions such as antibody activity, reaginic activity, etc. To further understanding of the functional significance of the classes and subclasses of immunoglobulins use will be made of "experiments of nature" leading to disease states characterized by selective immunoglobulin deficiencies. Patients with these diseases will be studied for functional aberrations of their immune systems. In addition, the structure of amyloid, a protein deposited in the tissue of some patients with the above diseases, will be determined.

Methods Employed: Studies required isolation of immunoglobulins from normal serum and also from the serum and urine of patients with multiple myeloma and macroglobulinemia. Isolation and analytic techniques included ion exchange chromatography, gel filtration, preparative electrophoresis, immune precipitation in gel, autoradiography and analytic ultracentrifugation. Protein structural studies utilized paper chromatography, high voltage electrophoresis, the amino acid analyzer and the automatic amino acid sequencer. Ultracentrifugation, including both sedimentation and equilibrium technique, has been used to characterize several systems. Cell free immunoglobulin synthesizing systems have been generated.

Major Findings: A procedure was worked out in which biologically pure immunoglobulin L-chain mRNA was prepared from a mouse myeloma with the aid of antibodies to L-chains. It was based on the specific precipitation of polysomes carrying nascent L-chains under conditions where the mRNA retained its full biological activity. Since unique properties of the antigen or antibody were not required, it seems that the antibody approach employed here would serve as a general solution for the isolation of any mRNA from fully functional eukaryotic and prokaryotic cells.

The mRNA coding for a mouse immunoglobulin L-chain was prepared from MOPC-321 myeloma polysomes specifically precipitated with antibodies directed to L-chains, followed by chemical purification on oligo-dT-cellulose. The biological purity of the L-chain mRNA was calculated to be >95%. This value was based on the estimation of contamination by non-L-chain mRNA activities that were present in a large abundance in RNA preparations extracted from the total polysome population. A similar degree of purity was calculated from the extent of precipitation of myeloma and nonmyeloma polysomes with anti-L-chain and non-L-chain antibodies. Chemical purity (95%) was determined from the amount of rRNA in the mRNA preparation by scanning of appropriate gels. In a cell free system the purified mRNA directed the synthesis of two precursors heavier than L-chain by about 1300 and 4700 daltons. Sequence analysis of one of the precursors showed it to contain a run of extra 20 amino acid residues (6 of which are leucines) coupled to the N-terminal end of mature L-chain. Cell free products labeled with 10^{14}C -amino acids yielded 27 out of 28 expected L-chain tryptic peptides, and 4 additional peptides. Most probably the latter were derived from extra pieces in the precursors, and the apparent loss of one peptide was due to modifications at the N-terminus. The main fraction of L-chain mRNA was composed of two species of about 420,000 and 450,000 daltons. These molecules are much larger than that required to code for a mature L-chain (calcd. $\sim 250,000$). The additional nucleotide mass can be accounted for, in part for coding the extra piece ($\sim 50,000$) and in part for the polyadenylate moiety.

Significance to Biomedical Research and the Program of the Institute: The development of a more complete understanding of the operation and control of the immune system requires that the component parts of the system be identified as precisely as possible. In the past, this work has depended heavily upon the utilization of myeloma protein which can be isolated in a more

homogeneous state than their normal counterparts in normal human serum. The finding that certain myeloma-like proteins both have antibody activity and form crystals under certain circumstances, leads to the exciting possibility of obtaining crystalline antibody molecules and of being able, therefore, to determine the three-dimensional structure of antibody molecules. It is only when both three-dimensional and primary sequence information can be put together that it will be possible to fully understand the nature of the interaction between antibodies and their antigens.

The generation of molecular variability among antibody molecules is not understood. Many theoretical formulations have been proposed and some of these can be subjected to critical testing. The accumulation of information concerning the primary amino acid sequences of the variable regions of heavy chains from the different classes and subclasses of immunoglobulin molecules should establish further constraints on the types of theories that may legitimately be entertained.

Studies of messenger RNA in an immunoglobulin synthesizing system are important to permit direct determination of the number of gene copies present in germ line, as well as somatic cells.

Proposed Course: Attempts to identify crystallizable myeloma proteins with antibody activity will be continued. Additional studies on the structure and pathogenesis of amyloidosis will be pursued.

Honors and Awards: None

Publications:

Ein, D., Kimura, S., and Glenner, G. G. : An amyloid fibril protein of unknown origin: Partial amino acid sequence analysis. Biochem. Biophys. Res. Comm. 46: 498-500, 1972.

Morell, A., Skvaril, F., Steinberg, A. G., van Loghem, E., and Terry, W. D.: Correlation between the concentrations of the four subclasses of IgG and Gm allotypes in normal human sera. J. Immunol. 108: 195-206, 1972.

Polmar, S. H., Waldmann, T. A., Balestra, S. T., Jost, M. C., and Terry, W. D.: Immunoglobulin E in immunologic deficiency disease: I. Relation of IgE and IgA to respiratory tract disease in isolated IgE deficiency, IgA deficiency and ataxia telangiectasia. J. Clin. Invest. 51: 326-330, 1972.

Glenner, G. G., Ein, D., and Terry, W. D.: The immunoglobulin origin of amyloid. Amer. J. Med. 52: 141-147, 1972.

Ein, D., Kimura, S., Terry, W. D., Magnotta, J., and Glenner, G. G.: Amino acid sequence of an amyloid fibril protein of unknown origin. J. Biol. Chem. 247: 5653-5655, 1972.

Freedman, M. H., Guyer, R. B., and Terry, W. D.: Isolation and characterization of electrophoretically homogeneous rabbit antihaptene antibody populations. III. NH_2 terminal light chain sequence analyses of focused anti-p-azobenzenearsonate antibody fractions. J. Biol. Chem. 247: 7051-7061, 1972.

Polmar, S. H., Robinson, L. D., Jr., and Minnefor, A. B.: Immunoglobulin E in bronchiolitis. Pediatrics 50: 279-284, 1972.

Kimura, S., Guyer, R. B., Terry, W. D., and Glenner, G. G.: Chemical evidence for lambda-type amyloid fibril protein. J. Immunol. 109: 891-892, 1972.

van Boxel, J. A., Paul, W. E., Terry, W. D., and Green, I.: IgD bearing human lymphocytes. J. Immunol. 109: 648-651, 1972.

Waldmann, T. A., Polmar, S. H., Balestra, S. T., Jost, M. C., Bruce, R. M., and Terry, W. D.: Immunoglobulin E in immunologic deficiency disease. II. Serum IgE concentration of patients with acquired hypogammaglobulinemia, thymoma and hypogammaglobulinemia, myotonic dystrophy, intestinal lymph-angiectasia and Wiskott-Aldrich Syndrome. J. Immunol. 109: 304-310, 1972.

Glenner, G. G., Terry, W. D., and Isersky, C.: Amyloidosis: Its nature and pathogenesis. Seminars in Hematology X: 65-86, 1973.

Polmar, S. H., Waldmann, T. A., and Terry, W. D.: IgE in immunodeficiency. Amer. J. Path. 69: 499-512, 1972.

Glenner, G. G. and Terry, W. D.: The immunoglobulin origin of amyloid fibril protein. The Bruges Colloquium, in press.

Polmar, S. H., Waldmann, T. A., and Terry, W. D.: A comparison of three radioimmunoassay techniques for the measurement of serum IgE. J. Immunol., in press.

Terry, W. D., Page, D. L., Kimura, S., Isobe, T., Osserman, E. F., and Glenner, G. G.: Structural identity of Bence Jones and amyloid fibril proteins in a patient with plasma cell dyscrasia and amyloidosis. J. Clin. Invest., in press.

Waldmann, T. A., Polmar, S. H., and Terry, W. D.: Metabolism and levels of IgE in immune deficiency disease. Vero Beach Conference, in press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5002 (c)

Title: Genetic, Structural and Functional Characteristics of Immunoglobulins and Related Proteins

Principal Investigator: William D. Terry, M. D.

Section:

Lab/Branch: Immunology Branch

Goal: To study the genetics, biosynthesis and functional characteristics of immunoglobulins and relate them to molecular structure.

Approach: 1) Appropriate molecules are identified, isolated and characterized, including where appropriate, linear sequence studies and three-dimensional studies. 2) Systems are devised to study the cell free biosynthesis of immunoglobulin molecules.

Progress: Biosynthesis - Messenger RNA has been purified from myeloma cells, translated in a cell free system and the product partially sequenced, proving that light polypeptide chains are being produced.

Genetics - Correlation has been demonstrated between IgG subclass serum concentration and Gm allotype.

Function - 1) A relationship has been demonstrated between IgE levels and respiratory disease in patients with immunoglobulin deficiencies. 2) IgD has been detected on the surface of a much larger number of circulating WBC than was expected.

Structure - 1) Proof has been obtained that the partial amino acid sequence of amyloid protein is identical to the sequence of the Bence Jones protein in a patient with multiple myeloma and amyloidosis. 2) A new chemical form of amyloidosis has been identified and one preparation completely sequenced.

Significance for Cancer Research (NCP Objective 5 Approach 4)

A complete knowledge of the genetic, biosynthetic, functional and structural relationships of immunoglobulins should be of help in permitting manipulation of this system in the therapy of patients with malignant disease.

Level of Effort: Professional Man-Years: 1 1/8

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cellular Immunity I

Previous Serial Number: Same

Principal Investigator: John R. Wunderlich, M.D.

Other Investigators: Elizabeth Simpson, D.V.M., John S. Macdonald, M.D.,
G. Nicholas Rogentine, Jr., M.D., Lloyd K. Everson, M.D.,
Elaine Esber, M.D.

Cooperating Units: Stephen B. Howell, M.D., Laboratory of Cell Biology, NCI

Man Years:

Total:	6
Professional:	4
Other:	2

Project Description:

Objectives: The project has been directed at understanding and manipulating mechanisms of cell-mediated immune cytotoxicity. Long-range goals are: 1) to identify and characterize factors which influence induction, maturation, and expression of a cellular immune response, 2) to define changes in cellular immunity which occur in tumor bearing hosts, and 3) to find means of manipulating the cellular immune response for therapeutic purposes. Immediate goals during the past year have been to study development of cytotoxic immune cells in vitro.

Methods Employed: Direct cell-mediated cytotoxicity has been measured in vitro by release of ^{51}Cr from labeled target cells incubated with immune lymphoid cells for 2 to 4 hours. Immune cells are collected from spleen, marrow, lymph nodes, thymus, and peripheral blood. Target cells are provided by tumors, tissue culture lines, and lymphoid cells from spleens or lymph nodes. Immune cells from spleens and nodes have also been separated by a Ficol velocity sedimentation technique which segregates cells on the basis of size (Everson, Buell and Rogentine, J. Exp. Med. 137, 343, 1973). This procedure, which requires about 45 minutes, is done prior to testing cells for cytotoxic effector cell activity. Normal lymphoid cells from spleens or nodes have been sensitized in vitro using previously established Mishell and Dutton tissue culture techniques (J. Exp. Med. 126, 423, 1967). In one series of experiments

donors of lymphoid cells were pretreated with rabbit antimouse lymphocyte sera produced by the 2-pulse, Levey-Medawar technique (Ann. N.Y. Acad. Sci. 129, 164, 1966).

In independent experiments the Hellström microcytotoxicity test has been used to study the mechanism of target cell killing involved in this assay (Hellström, et al., Proc. Nat. Acad. Sci. 66, 66, 1970).

Major Findings:

Background: Last year we found that normal C57Bl/6 mice developed excellent cytotoxic cellular immunity against EL-4, a tumor originally derived from C57Bl mice, if EL-4 was precoated with the plant lectin Concanavalin A. On the other hand, hosts injected with untreated EL-4 failed to develop cytotoxic cellular immunity. An intriguing finding was the spleen cells from hosts injected with untreated EL-4 developed high levels of cytotoxic activity if the sensitized cells were further challenged with uncoated EL-4 in vitro. In fact, normal spleen cells from nonsensitized C57Bl/6 donors responded well to uncoated EL-4 in vitro. We concluded that host mechanisms which inhibit cytotoxic cellular immune responses in vivo could be overcome in vitro. Thus, this year we elected to further explore development of cytotoxic cellular immune responses in vitro.

In Vitro Cytotoxic Cellular Immune Responses Following Adult Thymectomy: Findings of Gershon (J. Immunol. 109, 238, 1972), Cantor and Simpson (in preparation) argue that lymphoid cells of normal donors have thymus-derived lymphoid cells which suppress cellular immune responses in vivo. Dr. Simpson, a visiting associate in the laboratory this year, has looked for in vitro evidence of suppressor cells. The following findings have developed. Adult thymectomy increases the ability of responder lymphoid cells from nonsensitized donors to generate cytotoxic effector cells in vitro. This result frequently occurs when nonsensitized spleen cells are immunized in vitro against strong transplantation barriers (H-2), weak transplantation barriers (non-H-2), and tumor associated antigens. Thus, in 14 of 18 experiments, nonsensitized BALB/c spleen cells demonstrated higher cell-mediated cytotoxic activity against H-2 different C57Bl/6 cells, if the donors had been thymectomized. Cytotoxic activity obtained in vitro with spleen cells from thymectomized donors was 10-40% higher than activity obtained with cells from nonthymectomized donors. In 4 of 4 experiments involving non-H-2 antigenic differences, BALB/c spleens developed cellular cytotoxic activity in vitro against DBA cells if the responding cell donors had been thymectomized. Responder cells from normal nonthymectomized donors developed little or no cytotoxic activity. In each of two experiments responder spleen cells from thymectomized BALB/c donors developed cytotoxic cell activity against syngeneic LSTRA tumor cells. No cytotoxic response against syngeneic tumor was seen if responder spleen cells were from nonthymectomized normal donors. Four additional points are noteworthy: 1) cytotoxic cells developed in vitro are anti- θ sensitive (AKR anti-C3H θ), 2) thymectomy improves the in vitro cellular immune response only if donors are thymectomized at least 2 weeks before spleens are removed for in vitro sensitization, 3) responder spleen cells from thymectomized hosts

develop better cytotoxic responses than cells from normal donors irrespective of the responder-stimulator ratio, and 4) cytotoxic responses of spleen cells from thymectomized donors against non-H-2 and tumor associated antigens are low, e.g., about 1/25 of the activity developed against H-2 antigens. These findings led Dr. Simpson to postulate that normal mouse spleen cells have short-lived thymus-derived suppressor cells. Thymectomy eliminates the source of these cells. Preexisting "suppressor" cells lose activity within 2 weeks because of their short life span. Preliminary results of reconstitution experiments show that spleen cells from ALS treated donors will reduce the response of spleen cells from thymectomized donors to H-2 antigens. Responses are reduced down to but not below levels of cytotoxic activity seen with spleen cells from nonthymectomized donors. These results argue 1) that spleen cells from thymectomized donors lack "suppressor" cells and that these suppressor cells can be supplied by spleen cells from ALS treated donors, and 2) that the suppressor cells are ALS insensitive and thus are nonrecirculating cells.

Effect of Antilymphocyte Serum on Cytotoxic Lymphoid Cells and "Memory" Cells: Further work with ALS by Dr. Simpson has demonstrated that cytotoxic spleen cells developed in situ are not affected by ALS injected subcutaneously 24 hours before in vitro testing. However, responder spleen cells from non-sensitized donors develop little, if any, cytotoxicity in vitro against H-2 antigens if donors are treated with ALS 24 hours prior to establishing the cultures. These results suggest the cytotoxic cells are nonrecirculating lymphocytes, but the nontoxic precursor lymphocytes are recirculating. Of note was the finding that memory cells--nontoxic, sensitized precursor lymphocytes--from 4-times hyperimmunized donors are not sensitive to ALS. These cells were immunized in vitro 1 week after the fourth in vivo challenge. Memory cells from 1-time immunized donors, stimulated in vitro 4 weeks after sensitization, were sensitive to ALS. Thus, recirculating characteristics of memory cells depend on the level of sensitization of the donor.

Gradient Fractionation of Cytotoxic Lymphoid Cells and Their Precursors: Earlier findings argue that presensitized hosts have humoral suppressor mechanisms, perhaps antigen-antibody complexes (Sjögren, et al., Proc. Nat. Acad. Sci. 68, 1372, 1971), which can reduce the cytotoxic cellular immune responses. Results summarized above argue that even normal nonsensitized hosts have cellular suppressor mechanisms which will reduce the cytotoxic cellular immune response. Thus gradient separation of lymphoid cells has been pursued in an attempt both to investigate the relationship between lymphoid cells involved in generating cytotoxic immune cells and to bypass suppressor mechanisms. The following findings have developed. Spleen cells from donors sensitized against allogeneic H-2 different cells can be partially separated according to function by velocity sedimentation--a cell size separating technique. Ficoll gradients have been divided into four fractions following cell separation. Spontaneously dividing spleen cells from sensitized donors are found only in the bottom fraction (fraction 1) where the largest cells segregate. Cytotoxic immune cell activity is enriched threefold above unfractionated cells in the second fraction. Memory activity for cellular cytotoxicity is enriched only in fractions 1 and 2. Of note is that the ability of sensitized cells to generate cytotoxic effector cells (memory) is at least 2 1/2 times

higher in fraction 1 than unfractionated cells. It is not yet clear whether gradient separation of cells enriches immune activity because of concentrating appropriate cell types, removal of suppressor cell types, or a combination of the two possibilities. Reconstitution experiments are in progress.

Mechanisms of Cell-Mediated Cytotoxicity in the Hellström Microcytotoxicity

Assay: Dr. Esber has asked the following question using the Hellström microcytotoxicity assay in a mouse model system. Is target cell destruction specific? BALB/c hosts have been sensitized with a syngeneic SV40 virus transformed tumor (Tu5) developed in Dr. Lloyd Law's laboratory. Immune cells show specific antitumor activity in the microcytotoxicity assay. BALB/c lymphoid cells from donors immunized against an allogeneic tumor (EL-4) are not cytotoxic for SV40 transformed BALB tumors. BALB/c anti-EL-4 is, however, cytotoxic for the BALB Tu5 tumor in the presence of normal C57Bl/6 node cells. The reaction is specific in that normal node cells from AKR or BALB/c mice will not promote cytotoxicity. These findings have been interpreted as follows. BALB anti-EL-4 cells react with target cells from C57Bl/6 lymph nodes. This reaction promotes nonspecific cytotoxicity and damage to the SV40 transformed BALB tumor. This finding is important because it illustrates a mechanistic difference between the rapid 4-6 hour ⁵¹Cr release cytotoxicity assays and the long-term (24-72 hour) microcytotoxicity assays. Rapid cell-mediated cytotoxicity assays are highly specific in target cell destruction.

Significance to Biomedical Research and Program of the Institute: Current evidence argues that many tumor bearing hosts respond immunologically to tumors. One reason that tumors are not rejected is that the immune response is inappropriate. Humoral blocking factors and "suppressor" lymphoid cells may diminish the function of cytotoxic effector mechanisms. Information gathered during the last year on suppressor cells in a mouse model system has at least two important messages regarding host-tumor immunologic relationships. First, suppressor cell function is diminished by adult thymectomy, and second, cytotoxic lymphoid cell responses are increased as a result of thymectomy irrespective of the tumor immunizing dose. The latter point is of particular interest in that tumor antigen doses within regional areas of the host lymphon may vary widely, and thus it is desirable to increase the host immune response to a wide range of immunogenic tumor doses. Three notes of caution need to be mentioned: 1) suppressor cells may play a beneficial role in controlling autoimmune disease, 2) adult thymectomy may remove primitive precursors to cytotoxic cells in addition to removing suppressor cells, and 3) this year's work involved in vitro development of immune responses; in vivo correlates are needed.

Proposed Course of Project: Work will continue along the following lines of investigation: further understanding of 1) requirements for development of cytotoxic immune cells, 2) mechanisms for suppression of the cellular immune response, and 3) methods for bypassing or eliminating host suppression of cellular immunity.

Honors and Awards: None

Publications:

Martin, W. J., and Wunderlich, J.: Immune response of mice to Concanavalin A coated EL-4 leukemia. J. Natl. Cancer Inst. Monogr. 35: 295-299, 1972.

Wunderlich, J., Martin, W. J., Macdonald, J. and Fletcher, F.: Functional capacity of cytotoxic lymphoid cells. J. Natl. Cancer Inst. Monogr. 35: 243-249, 1972.

Martin, W. J., Esber, E., and Wunderlich, J.: Evidence for the suppression of the development of cytotoxic lymphoid cells in tumor immunized mice. Fed. Proc. 32: 173-179, 1973.

Yust, I., Wunderlich, J., Mann, D. L., and Buell, D. N.: Cytotoxicity mediated by human lymphocyte dependent antibody in a rapid assay with adherent target cells. J. Immunol., in press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5003(c)

Title: Cellular Immunity I

Principal Investigator: John R. Wunderlich, M.D., Elizabeth Simpson, D.V.M.,
John S. Macdonald, M.D.

Section:

Lab/Branch: Immunology Branch

Goal: Study development and expression of cytotoxic cellular immune activity in animal model systems. Identify host mechanisms which suppress cellular immunity and find means to bypass suppression.

Approach: The effector stage of cytotoxic lymphoid cell activity has been monitored by release of ^{51}Cr from damaged target cells. These assays require 1-4 hours. Thus, cytotoxicity represents activity of preexisting effector cells. Development of cytotoxic effector cells has been studied by immunizing lymphoid cells in vitro utilizing the Mishell-Dutton technique. Parameters have been varied by thymectomizing cell donors, treating donors with anti-lymphocyte serum, and separating cells according to size on continuous Ficoll gradients.

Progress: Normal mice have "suppressor" cells which inhibit in vitro development of cytotoxic lymphoid cells. Suppressor cell activity is reduced by adult thymectomy of donors. Neither suppressor cells nor cytotoxic cells are sensitive in vivo to ALS. Thus, they are not recirculating cells. ALS sensitivity of precursors to cytotoxic cells varies, depending on presensitization of hosts. Several immune activities have been partially separated on continuous Ficoll gradients: cytotoxicity, "memory" for cytotoxicity, and cell division.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Methods are being identified for increasing the host cellular immune response, both in vivo and in vitro.

Level of Effort: Professional Man-Years: 4

Serial No. NCI-5004(c)

1. Immunology Branch
- 2.
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Regulation of Growth and Biosynthetic Activity of Cells of the Immune System

Previous Serial Number: Same

Principal Investigator: Donald N. Buell, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1 3/4
Professional:	3/4
Others:	1

Project Description:

Objectives: 1) The development of large scale, long term cultures of functionally capable immune cells. 2) Delineating the factors which play a role in control of growth and proliferation of cells of the immune system. 3) Understanding the mechanisms which regulate lymphocyte differentiation.

Methods Employed: Tissue culture of human lymphoid cells is an important part of this work. Immunofluorescent cytology and radioimmuno-electrophoresis are used to identify specific immunoglobulins produced. Rates of production and turnover are studied by continuous and pulse label incubations with radioactive amino acids, with analysis of incubation medium and cytoplasm using solid phase radioimmunoassay. Standard counting techniques are used to follow cell growth. Incorporation of specific labeled precursors followed by autoradiography and scintillation counting are used to study rates of RNA and DNA synthesis, and to determine cell cycle parameters. Synchrony of cell growth is achieved by double thymidine and thymidine-colcemid blockade. Rate zonal centrifugation is used to separate cells according to size, cell cycle position and functional properties. Special culture techniques are used to establish high density stationary phase cultures, which on resumption of exponential growth synchronously enter the G₁ phase of the cell cycle.

Freeze-thaw elution and detergent solubilization permit isolation of plasma membranes. Lactoperoxidase radioiodination is used to label cell

surface proteins in intact cells. Proteins for cell receptor binding studies are iodinated by the chloramine T method. Specific cell surface receptors are identified by measuring the binding of radiolabelled ligands. Altered cell growth conditions, temperature shifts, enzyme treatment and additions of specific drugs are used to investigate the turnover of membrane components.

Antibody secretion by individual cells of the functional murine myeloma MOPC-315 is measured by incorporating TNP coated sheep RBC's in a modified Jerne plaque assay. Hemagglutination with TNP-SRBC's, and direct binding of ^3H labeled TNP- ϵ -amino lysine, are used to quantitate antibody levels in serum and ascites fluid.

Major Findings: A number of human lymphoid cell lines were examined and found to vary significantly in the quantity of surface insulin and growth hormone receptors. Cell lines derived from Burkitt lymphoma biopsies were found to have striking deficits of these receptors compared to other human lymphoid lines studied. Insulin receptors are also lacking in human and murine myeloma cells. Friend virus induced murine leukemia cell lines have low levels of insulin receptors which are lost during induced erythroid differentiation. Human lymphoid cell lines of T (thymic derived) type have low levels of insulin receptors.

Cultured lymphoid cells were found to have the special property of spontaneously shedding cell surface components under appropriate culture conditions. Between 15-20% of radioiodinated cell surface protein is spontaneously released into the medium. One important component released is the insulin receptor. This has permitted, for the first time, the isolation of true water soluble insulin receptors without the use of detergents. Other surface markers are retained and unchanged by culture conditions under which insulin receptors are shed.

Modulation of cell surface receptors and other markers have been induced by a number of techniques. Insulin receptors disappear from the cell surface when cells are grown in excess insulin under conditions where no spontaneous shedding of the receptor is seen. Friend virus induced erythroleukemia cells in established culture undergo erythroid differentiation when exposed to DMSO (dimethyl sulfoxide). Insulin receptors are lost during this transformation. The low levels of insulin receptors in human T cell lines can be increased by treatment with certain polynucleotides and other T cell stimulating agents.

A technique for rate-zonal centrifugation of cultured cells in a Ficoll gradient was developed which permits separation of cells in the G_1 , S, and G_2 phases of the cell cycle without subjecting cells to chemical treatment or growth perturbation.

In a collaborative study it has been shown that certain cultured human lymphoid lines produce a new lymphokine which stimulates bone resorption by osteoclastic activation. This has been nicknamed OAF for osteoclast activating factor. Both dialyzable and non-dialyzable species have been seen.

A useful murine myeloma model system (MOPC-315) has been developed which will permit further investigations of the interrelationships of cellular proliferation and immunoglobulin gene expression during plasma cell differentiation. An increased efficiency of antibody production can be seen with a slowing of tumor growth. This appears to be associated with cells spending a greater part of their time in the functional, immunoglobulin synthesizing G₁ portion of the cell cycle. Studies indicate that a constituent of ascites fluid may be responsible for G₁ arrest and foster differentiation.

Several continuous cell lines of MOPC-315 have been established. These tissue culture lines remain tumorigenic and will permit parallel in vivo and in vitro manipulations.

Significance to Biomedical Research and the Program of the Institute:

- 1) Synchronization: Techniques now exist for preparing large quantities of synchronized cultured lymphoblasts by chemical treatment or by growth pattern alterations. Smaller quantities of synchronized cells can be obtained by gradient centrifugation from exponentially growing unperturbed cultures.
- 2) Growth Control: A totally in vitro investigation of the repressor mechanisms involved in turning off lymphoid cell proliferation, and also mechanisms involved in lymphocyte activation, is now possible using tissue culture cell lines.
- 3) Myeloma: In vivo growth of a murine myeloma appears to follow a pattern nearly identical to other transplantable tumors. The alterations of immunoglobulin gene expression seen during growth of these malignant cells will provide important information towards understanding their counterparts, the normal antibody producing cells.
- 4) Key questions in immunology and cell biology in general center upon the nature of the alterations in molecular activity of a cell attendant upon binding of a specific activator molecule to its cell surface membrane. The finding that some but not all lymphocytes possess inulin receptors may open up new directions of investigation in these areas.
- 5) It is clear that much of the regulation of activity of mammalian cells is mediated by binding of specific effector molecules by cell surface receptors. Loss of critical cell surface receptors, analogous to the deficits of insulin and growth hormone receptors in the Burkitt lymphoma derived cell lines, may be the explanation of neoplastic cell behavior.

Proposed Course of Project: Explore culture conditions that will increase rate for successful establishment of human cells in permanent tissue culture. Factors such as CO₂, nutrients, feeder layers, virus preparations, etc., will be investigated.

Develop cell separation techniques in order to obtain specific functional cells for establishment in long term culture and detailed study.

Develop culture techniques for maintaining fully functional cells in high density cultures in order to establish high level in vitro antibody and immune cell production.

Investigate the specific molecular events involved in the onset of immunoglobulin synthesis in synchronized cultures.

The effect of insulin on membrane-bound ATPase and adenyl cyclase activity will be compared in lymphocytes with and without insulin receptors.

Study the incidence of specific hormone receptors in normal and transformed cell lines.

Use cell lines grown in serum free medium to prepare purified OAF.

Further investigate the metabolism, turnover rate, biosynthesis, etc. of the lymphocyte insulin receptor.

Define the incidence of hormone receptors in thymic and bone marrow lymphocytes, their subpopulations and changes during blast transformation.

Honors and Awards: None

Publications:

Fahey, J. L., Buell, D. N., and Sox, H. C.: Proliferation and differentiation of lymphoid cells: Studies with human lymphoid cell lines and immunoglobulin synthesis. Ann. N. Y. Acad. Sci. 190: 221-234, 1972.

Gavin, J. R. III, Buell, D. N., and Roth, J.: Water soluble insulin receptors from human lymphocytes. Science 178: 168-169, 1972

Gavin, J. R. III, Gorden, P., Roth, J., Archer, J., and Buell, D. N.: Characteristics of the human lymphocyte insulin receptor. J. Biol. Chem. 248: 2202-2207, 1973.

Gavin, J. R. III, Mann, D. L., Buell, D. N., and Roth, J.: Preparation of solubilized insulin receptors from human lymphocytes. Biochem. Biophys. Res. Comm. 49: 870-876, 1972.

Everson, L. I., Buell, D. N., and Rogentine, G. N., Jr.: Separation of human lymphoid cells into G₁, S, and G₂ cell cycle populations by use of a velocity sedimentation technique. J. Exp. Med. 137: 343-358, 1973.

Smith, R. W., Terry, W. D., Buell, D. N., and Sell, K. W.: An antigenic marker for human thymic lymphocytes. J. Immunol. 110: 884-887, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5004 (c)

Title: Regulation of Growth and Biosynthetic Activity of Cells of the Immune System

Principal Investigator: Donald N. Buell, M.D.

Section:

Lab/Branch: Immunology Branch

Goal: The development of large scale, long term cultures of functionally capable immune cells.

Approach: Studies are centered on the regulation of growth and proliferation, gene expression and membrane function in cultured lymphoid cell lines. Both T cell and B cell type lines are available. These cultured lymphocytes serve as model systems for developing an understanding of the mechanisms which control lymphocyte proliferation. Specific self regulatory factors are sought. Since most regulation in eukaryotic cells seems to be directed by factors binding to specific surface receptors, such receptors are under active investigation.

Progress: Immunoglobulin gene expression has been related to the G₁ and early S phases of the cell cycle. Growth techniques have permitted the generation of large cultures of stationary phase lymphocytes which behave much like resting lymphocytes and can be induced to synchronously enter the cell cycle. Insulin and growth hormone receptors have been identified in lymphocytes. These specific receptors are absent or markedly reduced in Burkitt lymphoma cell lines.

Significance for Cancer Research (NCP Objective 2 Approach 5)

The alterations of immunoglobulin gene expression seen during growth of these malignant cells will provide important information towards understanding their counterparts, the normal antibody producing cells.

Level of Effort: Professional Man-Years: 3/4

Serial No. NCI-5006 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: 1) Transplantation Antigens
2) Acute Leukemia Associated Antigens
3) Characterization of Lymphocyte Dependent Antibody

Previous Serial Number: Same

Principal Investigator: Dean L. Mann, M.D.

Other Investigators: Israel Yust, M.D.

Cooperating Units: Brigid G. Leventhal, M.D., Medicine Branch, NCI

Man Years:

Total: 2 3/4
Professional: 1 3/4
Others: 1

Project Description:

Objectives: The objectives of the studies are threefold: 1. a) to purify human transplantation antigens; b) to chemically, genetically and immunologically characterize these antigens; c) use soluble HL-A alloantigens to raise allotypic antisera in heterologous species; d) to define and characterize cell surface components that function as antigen receptor sites and relate them to HL-A alloantigens. This work supports Objective No. 3, Approach No. 5. 2. a) Identification of an antigen associated with acute leukemia. b) Isolation and characterization of this antigen; c) use antigen to produce heterologous antiserums; d) identify factor(s) in serum of leukemic patients that inhibit and/or promote cell-mediated cytotoxicity, to infect non-human cell lines with murine leukemia viruses and identify new antigens that cross react with the human acute leukemia antigen. In conjunction with this study and based on previous observation, an immunotherapy program was initiated with Dr. Leventhal of the Medicine Branch, NCI. Patients with acute leukemia were injected with RAJI cells. This laboratory has been undertaking the assessment of the immunologic response of these patients to this immunization procedure. This work supports Objective 6, Approach 3. A serum component (antibody) mediating cellular cytotoxicity (LDA) will be identified and characterized. Patients with autoimmune disease and malignancies will be screened for the presence of this antibody. This work supports Objective No. 6, Approach 4.

Methods Employed: ^{51}Cr release and dye exclusion cytotoxicity tests (See Project Report NCI-5008) were used to detect alloantigens. Human cells were obtained from normal blood bank donors and from long term tissue culture. Antisera detecting genetically determined human leukocyte alloantigens were obtained as described in Project NCI-5008. Alloantigens in subcellular form were detected by a cytotoxic inhibition assay using the ^{51}Cr release from target cells and employing the human leukocyte typing antisera.

Human leukocyte alloantigens were prepared by graded hypotonic lysis of cell suspensions, recovery of lipoprotein membrane fragments and papain digestion of these fragments to yield a water soluble antigen. Further purification was done by CM-Sephadex chromatography, gel filtration chromatography, electrofocusing and acrylamide gel electrophoresis. A number of different techniques were employed to solubilize HL-A alloantigens. These included detergents, chaotropic agents and 3M KCl. The physical-chemical properties of the alloantigens were investigated.

To aid in studies of identification and isolation of HL-A alloantigens, heterologous antisera were raised by injecting purified HL-A alloantigens into rabbits. These antisera were evaluated for cytotoxicity to human lymphoid cells as well as for their ability to precipitate solubilized HL-A alloantigens. The antisera was absorbed with cells in attempts to raise specific allotypic antisera.

The acute leukemia associated antigen(s) was detected in dye exclusion and ^{51}Cr chromium release cytotoxicity assays. Antiserum used in these studies were obtained by immunizing rabbits with water soluble cell membrane preparations from the tissue culture cell line RAJI. Cell mediated cytotoxicity studies were performed by the method described by Wunderlich. Peripheral blood lymphocytes from patients with acute leukemia were labeled with ^{51}Cr , incubated with autologous sera or serum from other leukemic patients and/or with heterologous antiserum that detect the acute leukemia antigen. Normal peripheral blood lymphocytes were added and the lysis or inhibition of lysis mediated by the various sera determined. Patients injected with the RAJI cell lines were assessed for the development of cytotoxic lymphocytes, LDA, and complement dependent cytotoxic antibody. Serum from these patients were screened for substances that blocked cytotoxic reactions.

Sera were obtained from patients who had received multiple transfusions or had a diagnosis of autoimmune disease or malignancies. These sera were tested in the cell-mediated cellular cytotoxicity reaction using various target cells and normal peripheral blood lymphocytes. The sera tested from the various patients were screened for their ability to mediate the cellular cytotoxicity reaction (LDA). The positive sera were fractionated by gel filtration, ion exchange chromatography, and electrofocusing. These fractions were tested for LDA to determine the immunoglobulin component responsible for this antibody activity.

Major Findings: Several methods were used to prepare soluble cell membrane antigens (HL-A) from tissue culture cell sources as well as from peripheral blood lymphocytes, particularly from patients with acute leukemia. Membrane materials solubilized with NP-40 were fractionated to ascertain the molecular characteristics. Immune precipitation tests were developed and used to isolate the HL-A containing membrane substances. The antigen-antibody complexes were dissociated and the antigen characterized on disc gel electrophoresis.

Antiserums obtained from rabbits immunized with the soluble cell membrane component from the cell line RAJI were found to detect an antigen which is associated only with acute leukemia cells. Peripheral white blood cells from patients in remission and from normal peripheral blood lymphocytes could not be demonstrated to contain this antigen. Cultured cell lines derived from various neoplasms were also tested for the presence of this antigen and found to be negative. This antigen could be demonstrated to be induced in human embryonic kidney cell lines coincident with infection with murine Rauscher leukemia virus.

In the studies investigating LDA and serum blocking factors in serums from acute leukemia patients, specific immune reactivity was demonstrated in approximately one-third of the patients. However, in the majority of the patients tested thus far it appears that the serum contains a component which inhibits the reactivity of the LDA activity of heterologous antisera. Other investigators have demonstrated blocking factors in neoplastic disease which may explain the lack of immune reactivity of patients against their developing neoplasms. It appears that there is a blocking factor also in patients with acute leukemia. These studies have been extended to the examination of immune reactivity of leukemia patients immunized with the RAJI cell line. Blocking factors have been demonstrated in the serums of patients during relapse.

In studies attempting to isolate and characterize LDA it has been demonstrated that patients receiving multiple transfusions have highest titers of this serum component. These sera were fractionated by gel filtration and ion exchange chromatography as well as electrofocusing. When these fractions were tested it was found that the maximum LDA activity did not correspond with maximum protein concentrations of IgM, IgA, IgG. These findings suggest that the LDA may be a subclass of IgG, or indeed, may be a new class of immunoglobulins. Additional studies have indicated that cytotoxic antibody can be separated from LDA.

Significance to Biomedical Research and the Program of the Institute: The preparation and analysis of soluble human transplantation antigens has both fundamental and clinical importance. Information gained as to the character of these antigens and the nature of the integration of these materials into the cell membranes structures will add to our knowledge of genetic

control as well as fundamental knowledge in basic cell membrane structure. In a clinical situation these soluble transplantation antigens could be used to induce tolerance or enhancement of allografted tissue and thus obviate the need for hazardous immunosuppressive agents.

The demonstration that acute leukemia has associated specific antigen or antigens is fundamental to understanding etiology and possible therapeutic approaches to treatment of this disease. The appearance of neo-antigens on cell surface has been well demonstrated in a variety of tumor systems in lower animals.

The demonstration that acute leukemia cells possess what appear to be specific antigenic determinants may allow a search for an etiologic agent which will cause this antigen to appear on acute leukemia cells. Immunotherapy has been proposed in the acute leukemias. Such a program implies that acute leukemia cells do possess a particular antigen, which is shared by the cells from a number of patients with this disease. Our demonstration that there is indeed an acute leukemia associated antigen makes more rational the treatment of this disease by these means. The demonstration that there are substances in the acute leukemia serum which either block LDA activity or mediate such activity suggests a fine balance between immunity and "tolerance" in the patients with this disease. On the basis of the above observation and immunotherapy program has been initiated.

The isolation and characterization of the lymphocyte dependent antibody is a relatively new area for exploration. The LDA appears to be an antibody which is not correlated with complement dependent cytotoxic serum components and may thus represent a new mechanism for destruction of tissues by the host's own lymphocytes. Investigation of the nature of this antibody and its specificity of reaction should contribute to our knowledge of immune reactions in patients with autoimmune disease as well as those individuals who possess malignancies.

Proposed Course of Project: Further chemical and biologic characterization of soluble HL-A alloantigens will be undertaken. Studies will be designed to isolate the antigenic determinants by immune precipitation techniques and to characterize this isolated product. Studies will be continued testing solubilized histocompatibility antigen for possible use in clinical situations for the induction of tolerance and/or enhancement.

Studies will continue in the isolation and characterization of the antigen associated with acute leukemia cells. In addition, efforts are currently being made to screen a number of the known oncogenic RNA viruses to attempt to correlate infection with these viruses and the appearance of antigen on cell surfaces. The various factors in the serum from leukemic patients which are either blocking or enhancing cell mediated cytotoxicity reactions will be isolated and characterized.

Further studies will be undertaken in an attempt to identify the specific serum component responsible for the LDA activity.

Honors and Awards: None

Publications:

Merrit, C. B., Mann, D. L., and Rogentine, G. N., Jr.: Cytotoxic antibody in human graft versus host disease. Nature 232: 638, 1971.

Mann, D. L.: Comparison of HL-A alloantigen solubilized by papain and TIS. In Transplantation Antigens, B. D. Kahan and R. A. Reisfeld, (eds.) Academic Press, New York and London, 1972. pp. 287-297.

Mann, D. L.: Effect of enzyme inhibition on the solubilization of HL-A antigen with 3M KCl. Transplantation 14: 398-401, 1972

Halterman, R., Leventhal, B. G., and Mann, D. L.: An acute leukemia associated antigen: Clinical correlation. New Eng. J. Med. 287: 1272-1274, 1972.

Gavin J. R., Mann, D. L., Buell, D. N., and Roth, J.: Preparation of solubilized insulin receptors from human lymphocytes. Biochem. Biophys. Res. Comm. 49: 870-876, 1972.

Mann, D. L., Halterman, R., and Leventhal, B. G.: Cross reactive antigens on human cells infected with Rauscher leukemia virus and on human acute leukemia cells. Proc. Natl. Acad. Sci. 70: 495-497, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5006 (c)
Title: Transplantation Antigens

Principal Investigator: Dean L. Mann, M. D.
Section:

Lab/Branch: Immunology Branch

Goal: The goal of this project is to solubilize, isolate, serologically and biochemically define human transplantation antigens.

Approach: Membranes from human lymphoid cells in long term tissue culture are analyzed by cytotoxicity assays for the presence of HL-A alloantigens. The membranes are isolated and the HL-A alloantigens are then solubilized by papain or detergents. The solubilized products are purified using Sephadex and ion exchange chromatography, electrofocusing, and acrylamide gel electrophoresis. The solubilized materials are assayed for antigenic activity using inhibition of cytotoxicity as well as immunoprecipitation techniques. The immunoprecipitation is either by direct precipitation using heterologous antisera prepared against the isolated membrane components, or an indirect assay using human alloantisera which is then precipitated by anti-immunoglobulins.

Progress: Heterologous antisera having precipitating antibodies to the membrane components bearing the HL-A alloantigens have been found to directly precipitate cell membrane components. Dissociation of the antigen-antibody complexes yielded an antigenic product which can be characterized as to size. The approximate molecular weight of this material is approximately 60,000. Using the indirect precipitation technique, antiserum defining specific HL-A alloantigenic determinants have been used to compare with the materials precipitated by the heterologous antisera. The product resulting from the dissociation of the antigen-antibody complexes after indirect precipitation has approximately the same size as the material isolated by the direct precipitation with the heterologous antisera.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Immunotherapy of cancer is predicated on the presence of tumor specific antigens. The above project relates the identification of these tumor specific antigens by using the prototype of the transplantation antigens for developing techniques to isolate and characterize tumor specific antigen.

Level of Effort: Professional Man-Years: 1 3/4 (Parts 1, 2, 3 inclusive)

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5006 (c)

Title: Acute Leukemia Associated Antigens

Principal Investigator: Dean L. Mann, M. D., Brigid G. Leventhal, M. D.
Section:

Lab/Branch: Immunology Branch and Medicine Branch

Goal: The goal of this project is to identify and isolate an antigen associated with acute leukemia and to use a long term tissue culture cell line bearing this antigen to immunize patients with acute leukemia. Assessment of immunologic response of these leukemic patients to the immunizing material is being made.

Approach: An antigen or antigens on acute leukemia cells have been identified using heterologous antisera raised against the RAJI lymphoid tissue culture cell line. This antigen has been identified by complement dependent cytotoxicity assays. Attempts have been made to isolate this antigen and to separate it from the HL-A alloantigens using papain and/or detergent solubilization techniques. The presence of the antigen is determined by cytotoxicity inhibition assays. The RAJI cell line has been employed to immunize patients with acute leukemia. The immunization procedure follows a course in chemotherapy. 5×10^8 cells are given subcutaneously in three injections ten days following the last dose of chemotherapy. The patients' sera before and after immunization are tested for either lymphocyte dependent or cytotoxic antibody to the immunizing cell as well as to the patients' frozen autochthonous tumor cells.

Progress: Antigenic material which specifically inhibits the antisera detecting the acute leukemia associated antigen can be separated from the major HL-A alloantigens by DEAF Sephadex chromatography after papain solubilization. This material has been used to attempt to develop more specific antisera for detecting the presence of the acute leukemia antigen. Two to four patients immunized with the RAJI cell lines have developed cytotoxic antibody to the RAJI cell line as well as to allogeneic acute leukemia cells. It has been found that during a period of relapse in the acute leukemia disease there is a serum factor which inhibits the cytotoxicity antisera directed against the acute leukemia cells. Attempts are being made to elucidate the nature of this inhibiting factor.

Significance for Cancer Research (NCP Objective 6 Approach 1)

The identification of acute leukemia associated antigen has allowed an approach to immunotherapy of this particular disease.

Level of Effort: Professional Man-Years: 1 3/4 (Parts 1, 2, 3 inclusive)

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5006 (c)

Title: Characterization of Lymphocyte Dependent Antibody

Principal Investigator: Dean L. Mann, M. D. and Israel Yust, M.D.

Section:

Lab/Branch: Immunology Branch

Goal: The goal of this research project is to identify and characterize an antibody mediating cytotoxicity (LDA) and to screen patients with malignancies for the presence of this antibody.

Approach: Human lymphocyte dependent antibody has been obtained from patients who have been multiply transfused. The presence of this antibody is detected by incubating the serum with ^{51}Cr labeled target cells and the addition of lymphocytes. The presence of this antibody is determined by the release of ^{51}Cr from the target cells. The nature of this lymphocyte dependent antibody has been investigated by separation of the immunoglobulin by the usual procedures, i.e., Sephadex chromatography and ion exchange chromatography, as well as isoelectrofocusing. Isoelectrofocusing experiments are being employed to attempt to separate complement mediated cytotoxic antibody from the lymphocyte dependent antibody.

Progress: During the past year it has been demonstrated that the lymphocyte dependent antibody can be identified in patients that have been multiply transfused. This antibody seems to develop in part against components on cell surfaces which are non-HL-A in their specificity and reactivity. This antibody has been found to be associated with the immunoglobulin G fraction using the usual serum protein separation techniques. However, employing isoelectrofocusing, the complement dependent cytotoxicity component of the serum can be separated from the lymphocyte dependent antibody component. The nature of this particular component is being elucidated.

Significance for Cancer Research (NCP Objective 6 Approach 4)

The demonstration that a component of human serum mediates lymphocyte dependent cytotoxicity is a relatively new finding. The elucidation of the nature of this component and its participation in the rejection of either transplanted tissues and/or tissues undergoing neoplastic transformation remains to be elucidated. Pursuing this goal will help us understand the immunologic response of individuals to foreign materials, including cells that have undergone neoplastic transformation.

Level of Effort: Professional Man-Years: 1 3/4 (Parts 1, 2, 3 inclusive)

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Preclinical Immunological Studies in Hematopoietic Cell Transplantation.

Previous Serial Number: None

Principal Investigator: G. Nicholas Rogentine, Jr., M.D.

Other Investigators: John R. Neefe, M.D.

Cooperating Units: C. C. Darrow, D.V.M., Litton Bionetics

Man Years:

Total: 1 1/4

Professional: 1 1/4

Other: 0

Project Description:

Objectives: To investigate newer forms of immune suppression of allo-graft rejection and the graft-versus-host reaction (GVHR) in a rhesus monkey bone marrow transplant model prior to clinical use, 2) To identify monkey transplantation antigens, 3) to use knowledge obtained in 2) to select appropriate donor recipient combinations so that the monkey model can even more closely approximate the human situation; 4) To establish a colony of pedigreed rhesus families to study the genetics of transplantation antigens and to eventually provide a source of related donor-recipient pairs for bone marrow transplantation.

Almost all of this work is being conducted by a close collaboration with Litton Bionetics under contract NIH-NCI-G-70-2103. Dr. Darrow is the Principal Investigator and Dr. Rogentine the Project Officer. Dr. Neefe is the Project Officer's principal NCI collaborator.

Methods Employed: Experiments done in the past 12 months have employed a variety of techniques and methods. Femoral bone marrow obtained by aspiration from anesthetized donors is administered intravenously to lethally X-irradiated recipients. Such allografted monkeys are then medically supported and observed for the remainder of their life with frequent measurement of a number of clinical, biochemical, and immunologic parameters. In some cases frozen autologous marrow has been given. Lymphocyte antigens of the Rhesus monkey are studied by standard eosin dye lymphocytotoxicity tests employing

Rhesus alloantisera obtained from planned immunizations with skin grafts and leukocyte injections. The mixed leukocyte culture technique was adapted for use with monkey leukocytes and provides another method of assessing histocompatibility.

The production of anti-rhesus lymphocyte sera (ALS) has continued with the immunization of rabbits with purified rhesus thoracic duct lymphocytes. The ALS resulting from these immunizations has been tested for activity in vitro, and for immunosuppressive properties in vivo using rhesus skin graft and GVH systems. Some of this ALS was purified by adsorption to and elution from a thymocyte stroma column.

Major Findings: The major findings of the past year are well detailed in the contractor's Progress Report dated March 1973. We will summarize these findings:

1. Newer Modes of Immune Suppression of GVHR

A model of bone marrow transplantation between unrelated rhesus monkeys had previously been established in which the graft-versus-host reaction (GVHR) is uniformly fatal in less than 17 days. Rabbit anti-rhesus-thoracic-duct-lymphocyte serum (ALS) had previously been used (Project Reports [NCI-5011, 1970-1972]) to modify the GVHR and produced prolonged survival. In the current contract year the result was reproduced with two new pools of ALS. The correlation between the skin graft assay of ALS and the efficacy of ALS in diminishing GVHR was improved.

The important role of immune deficiency in the death of animals thought to be suffering chronic GVHR was emphasized in a histopathologic review of transplants from 1970 to 1972.

Use of purified ALS eluted from a thymocyte stroma immunoadsorbent column and use of post-grafting ALS did not improve survival in GVHR.

2. Rhesus Histocompatibility Antigens

Definition of the RhL-A system was improved. Analysis of data from the typing of 258 unrelated monkeys resulted in (a) confirmation of five previously well defined antigens; (b) improved definition of two antigens; (c) definition of two new antigens; (d) demonstration of the inclusion of Be 7 in Be 3 and of Be 5 in Be 12; (3) provisional partition of the antigens among two segregant series: Be-1, -2, -5, -12, and Be-3, -6, -7, -10.

The mixed leukocyte culture reaction has been established and studies of RhL-A matched siblings indicate the rhesus MLC locus is separable from the RhL-A locus.

3. Histocompatibility and Bone Marrow Transplantation

The first demonstration of a beneficial influence of RhL-A matching upon bone

marrow transplantation between unrelated primates was derived from transplant data through November, 1972. Matched recipients survived a median of 35 days whereas mismatched recipients survived only 14 days. ALS was used in all the transplants.

4. Establishment of a Breeding Colony

A colony of over twenty family groups of parents and full siblings was established, and the first new births and sib-sib matings were accomplished.

Significance to Biomedical Research and the Program of the Institute:

The National Cancer Institute has made a strong commitment to a clinical program of marrow transplantation. The first stage of this program is a reality. However, only HL-A matched donors have been used. This greatly limits the application of this form of therapy, as well over 60% of all potential recipients do not have matched donors. Transplantation of mismatched marrow has almost invariably resulted either in no graft take or fatal GVHR and even in perfectly matched donor-recipient combinations fatal GVHR may occur, although at a lower frequency. Our recent findings with respect to the modification of GVHR with ALS and RhL-A matching provides tools for study of the basic mechanism of GVHR and also may be applicable clinically, particularly in situations where it is necessary to transplant marrow between individuals not perfectly matched at HL-A. An understanding of the pathophysiology of GVHR obtained in this system will also be of benefit in planning new methods of intervention in this problem as it occurs in man. Marrow transplantation may have a role in tumor immunotherapy but understanding of GVHR is certainly a prerequisite to this.

Proposed Course of Project: With respect to basic questions in transplantation, we will attempt to understand the pathophysiology of GVHR, as well as the mechanism by which ALS acts to modify the reaction. This will involve a number of observations comparing clinical, histopathological, and immunological data from ALS treated and untreated animals. Other methods will be used, either alone or in combination, in an effort to further control the GVHR reaction. These will include further studies with soluble lymphocyte antigens in attempts to produce tolerance, and the use of blocking antibody to modify the reaction. The possibility of using nonlethal forms of immunosuppression to prepare the recipient for transplant will also be considered (such as the use of ALS and sublethal doses of X-ray).

Further studies of monkey leukocyte antigens will be conducted using lymphocytotoxicity. New antisera will be produced by cross immunizing antigen matched monkeys. Special emphasis will be placed upon typing of families. Mixed leukocyte cultures will also be carried out in these families. The relationship of leukocyte antigens to graft success will be studied. The pedigreed colony will be enlarged by addition of new female breeders and any natural births.

Honors and Awards: None

Publications:

Merritt, C. B., Darrow, C. C. II, Vaal, L. A., and Rogentine, G. N., Jr.: Bone marrow transplantation in rhesus monkeys following irradiation: Modification of acute graft versus host disease with antilymphocyte serum. Transplantation 14: 9-20, 1972.

Merritt, C. B., Darrow, C. C. II, Vaal, L. A., Herzig, G. P., and Rogentine, G. N., Jr.: Rescue of rhesus monkeys from acute lethal graft versus host disease using cyclophosphamide and frozen autologous bone marrow. Transplantation 15: 154-159, 1973.

Neefe, J. R., Vaal, L., Darrow, C. C. II, and Rogentine, G. N., Jr.: Mixed lymphocyte reactivity in rhesus sibships. Transplantation, in press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5011 (c)

Title: Preclinical Immunological Studies in Hematopoietic Cell Transplantation

Principal Investigator: G. Nicholas Rogentine, Jr., M.D., John R. Neefe, M.D.
Section:

Lab/Branch: Immunology Branch

Goal: The goal of this project is to study newer methods of obtaining allografts and of modifying graft versus host disease in bone marrow transplantation in a rhesus monkey pre-clinical model.

Approach: Lethally irradiated monkeys are given allogeneic marrow 24 hours later. Some recipients also get rabbit anti rhesus thoracic duct lymphocyte sera at the time of radiation. Animals were followed clinically, bacteriologically, and chemically until death. Autopsies were performed.

Rhesus histocompatibility antigens (RhL-A) were assayed by a lymphocyte microcytotoxicity test. Unrelated and related monkeys were tested. Donors and recipients for transplantation were picked on the basis of RhL-A matching.

Progress: ALS given 24 hours before allografting could significantly prolong survival and delay the onset of graft versus host disease. This was even more marked if the donor and recipient were matched for RhL-A antigens (Matched had a median survival time of 35 days, mismatched 14 days). Various post grafting schedules of ALS (in addition to pre-graft ALS) did not yield any additional prolongation of survival.

RhL-A antigen analyses revealed the existence of 8 antigens which fitted into two closely linked segregant series of 4 antigens each.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Hematopoietic support for patients undergoing massive chemo- or radio-therapy may require bone marrow transplant. Moreover, the graft versus host reaction eventually may be useful in tumor immunotherapy; i.e., as a graft versus tumor reaction.

Level of Effort: Professional Man-Years: 1 1/4

Serial No. NCI-5014(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Lymphocyte Stimulation: The Chemical Basis of Gene Activation
in Cellular Immunity

Previous Serial Number: Same

Principal Investigator: Ronald H. Schwartz, M.D.

Other Investigators: Barry S. Handwerger, M. D., Bilha Schechter, Ph.D.,
N. Franklin Adkinson, M.D., William D. Terry, M.D.

Cooperating Units: None

Man Years:

Total: 3 7/8
Professional: 2 7/8
Other: 1

Project Description:

Objectives: 1) To chemically define the molecules involved in the early stages of lymphocyte activation in order to gain an understanding of the nature of the response of the sensitized lymphocyte to antigen. 2) To utilize these findings to develop sensitive assays for cellular immunity in rodents and man. 3) To establish the nature of the cells involved in these assays.

Methods Employed: Experimental animals are immunized with protein antigens and serve as the source of lymphocytes for many of these studies. Mouse spleen cells and human peripheral blood cells are also used. The stimulation of protein synthesis by mitogens, specific antigen, or in mixed leukocyte interactions is determined by measuring the incorporation of radioactive leucine into cellular protein. Identification of chemical changes in proteins after lymphocyte activation has been measured using a double-label technique in the presence and absence of antigen or phytohemagglutinin. Lymphocyte proteins are then fractionated using gel filtration chromatography and polyacrylamide gel electrophoresis.

Major Findings: During the past year, we have been evaluating the SPS assay system as an in vitro measure of mouse lymphoid cell responsiveness to specific (antigen and MLC) and nonspecific (mitogens) stimuli. In particular, we addressed ourselves to the question: Which type of lymphoid cell responds

to these stimuli, the thymus derived (T) or the bone marrow-derived (B) lymphocyte, or both?

Our approach to the problem has been to study the responsiveness of purified T and B cell preparations. These have been obtained in a variety of ways. Antibodies against the theta-C₃H alloantigen were raised in AKR mice and used to deplete BALB/c spleen and lymph node cells of T lymphocytes by complement-mediated cytotoxicity. B cell enriched spleens were also prepared by thymectomy, lethal irradiation, and bone marrow reconstitution of young adult mice.

Purified T lymphocytes were obtained by using cortisone-resistant thymocytes and by passage of peripheral lymphoid cell suspensions over columns containing plastic beads coated with antigen-antibody complexes. B lymphocytes, which contain a surface receptor for Ag-Ab complexes, were specifically removed by binding to these columns.

Thus far, we have demonstrated that purified T cell populations can respond with increased protein synthesis to PHA and Con A as well as to allogeneic cells in mixed lymphocyte culture. We are presently evaluating the responsiveness of purified T cells to specific antigens and of purified B cells to mitogenic and antigenic stimuli.

Since most observations in the literature relative to in vitro lymphocyte transformation have been made using DNA rather than protein synthesis as a measure of stimulation, we developed a serum-free, microtiter DNA assay system to be run in parallel with the SPS system. In this way, our results could be more easily compared to those in the literature. Although in most cases the DNA and protein systems have shown comparable results, it should be remembered that they are measuring different biochemical events and, thus, may occasionally show some disparity. An example of this was found in the stimulation of normal BALB/c thymocytes by PHA and Con A. In the DNA system the response to Con A was at least 10-fold greater than the response to PHA, whereas in the protein system the responses were equivalent. The significance of this finding is at present being investigated.

Significance to Biomedical Research and the Program of the Institute: Cell-mediated immune responses play an important role in the host response to tumors and transplanted tissue. Few methods currently exist for assaying these cellular immune responses, and the assay we have described should be a valuable one for this purpose. The speed, convenience, and reproducibility of the assay are assets that should prove useful for clinical studies. Because it measures the very early events in the activation of the lymphocyte, this assay might detect phenomena not theretofore detectable by other means.

The mechanism of the activation of immune lymphocytes represents an important gap in knowledge of the immune system. The molecules we are isolating from activated lymphocytes in our double-label studies may play an important role in the process of gene activation in the lymphocyte. The stimulation of lymphocytes by nonspecific mitogens and by specific antigens is also an excellent model for the study of gene activation and control, and thus

parallels can be drawn to the regulation of gene function in differentiation, malignant transformation, and other cellular phenomenon. By studying the early protein changes associated with lymphocyte activation, we hope to gain insight into the general phenomenon of gene activation in mammalian cells.

Proposed Course of Project: We are currently trying to extend to humans the assay we have developed for measuring cellular immunity. This assay will be used to detect and isolate tumor and histocompatibility antigens as well as to evaluate the host response to these antigens.

Honors and Awards: None

Publications:

Rosenberg, S. A. and Levy, R.: Synthesis of nuclear proteins by lymphocytes within minutes after contact with phytohemagglutinin. J. Immunol. 108: 1105-1109, 1972.

Levy, R. and Rosenberg, S. A.: The early stimulation of protein synthesis in sensitized guinea pig lymph node cells by antigen. J. Immunol. 108: 1073-1079, 1972.

Rosenberg, S. A. and Levy, R.: A rapid assay of cell mediated immunity to soluble antigens based on the stimulation of protein synthesis. J. Immunol. 108: 1080-1087, 1972.

Rosenberg, S. A., Levy, R., Schechter, B., Ficker, S. and Terry, W. D.: A rapid microassay of cellular immunity in the guinea pig and mouse. Transplantation 13: 541-545, 1972.

Schechter, B., Rosenberg, S. A., Levy, R., Ficker, S., and Terry, W. D.: Early evaluation of mixed leukocyte interactions in mice and guinea pigs using measurements of protein synthesis. Cellular Immunol. 6: 261-272, 1973.

Levy, R., Levy, S., Rosenberg, S. A., and Simpson, R. T.: Selective stimulation of nonhistone chromatin protein synthesis in lymphoid cells by phytohemagglutinin. Biochemistry 12: 224-228, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5014(c)

Title: Lymphocyte Stimulation: The Chemical Basis of Gene Activation in Cellular Immunity

Principal Investigator: Ronald H. Schwartz, M.D.

Section:

Lab/Branch: Immunology Branch

Goal: To chemically define molecules involved in the early stages of lymphocyte activation, 2) to utilize these findings to develop sensitive assays for cellular immunity, and 3) to define the cell populations involved in these responses.

Approach: 1) Sensitized lymphoid cells are stimulated in vitro and chemical changes measured. 2) A specific assay based upon stimulation of protein synthesis has been utilized and the responsive cell populations evaluated by cell fractionation techniques and killing of specific subpopulations of lymphoid cells.

Progress: 1) Purified T cells respond with increased protein synthesis to stimulation by mitogens and allogeneic cells. Response of purified B cells is under investigation. 2) A serum-free microtiter lymphocyte culture system using DNA synthesis to monitor stimulation has been devised.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Tests capable of monitoring cellular immune responses to tumor antigens are needed to monitor immunologic responsiveness of tumor-bearing hosts. Knowledge of mechanisms involved in the activation of lymphocytes are important in ultimately being able to manipulate the immune system.

Level of Effort: Professional Man-Years: 2 7/8

Serial No. NCI-5016(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cellular Immunity II

Previous Serial Number: Same

Principal Investigator: John R. Wunderlich, M.D.

Other Investigators: Dean L. Mann, M.D.

Contractor: Litton-Bionetics Research Laboratory

Contract NIH-NCI-G-70-2207

This progress report represents only a portion of work supported by contract NIH-NCI-G-70-2207

Cooperating Units: Michael Blaese, M.D., Metabolism Branch, NCI;
John Potter, M.D., Georgetown University

Man Years:

Total: 1 1/2
Professional: 1/2
Others: 1

Project Description:

Objectives: This project, which was initiated in 1970, has been directed primarily at understanding cell-mediated immune cytotoxicity in humans. The long-range goals are: 1) to identify human cells capable of destroying foreign cells by cytotoxic, cell-mediated immune reactions, 2) to characterize factors which influence development of cellular immune effector cells, 3) to characterize the mechanism of foreign cell destruction by effector cells, and 4) to define changes occurring in the cellular immune cytotoxic responses which accompany immune deficiency diseases, malignancies, transplanted organs, autoimmune states, and iatrogenic immune suppression.

A year ago the project was expanded to include animal model systems directly relevant to the human work. The goals of the animal model system work are: 1) to develop tumor specific heterologous lymphocyte dependent antibody and assess its antitumor effects in situ, and 2) to determine if syngeneic tumors grow because of an inadequate or inappropriate response.

Methods Employed: 1) Human Direct, Cell-Mediated Immune Cytotoxicity. This is measured by the release of ^{51}Cr from labeled target cells incubated with immune lymphoid cells for 4 hours. Details of the assay utilizing human cells have been described elsewhere (Wunderlich, et al., Transplantation 13: 31, 1972). Target cells are prepared from peripheral blood, pathological fluid exudates, tissue culture lines, and biopsy specimens of lymph nodes and solid tumors. Solid tumor samples and cells adhering to tissue culture bottles are mechanically separated. Aggregated cells are allowed to settle. Red cells are then removed by ammonium chloride Tris lysis. The resulting cell suspension is labeled with ^{51}Cr . Normal and immune lymphoid-attacking cells are prepared primarily from human peripheral blood. Heparinized peripheral blood is passed through nylon columns to remove polymorphonuclear cells and monocytes and then settled in plasmagel to reduce the red blood cell content. Recent studies of multiply transfused humans has shown that cytotoxicity in this assay is specific for direct cell-mediated reactions and independent of conventional complement humoral antibody and nonspecific toxins.

2) Human Lymphocyte Dependent Antibody (LDA) Mediated Cytotoxicity. This is also measured by a chromium release assay. After ^{51}Cr labeling, target cells are washed by centrifugation and then incubated with heat inactivated serum or plasma containing LDA for 1/2 hour. Incubation mixtures include carrageenan, a C' attachment inhibitor. Target cells are again washed and then incubated with nonsensitized attacking lymphoid cells for 4 hours.

3) In Vitro Immunization of Human Peripheral Blood Lymphocytes. Human lymphocytes have been immunized against allogeneic cells using the procedures of Miggianno and his colleagues (Transpl. Proc., in press). This procedure has been modified by adding in vitro immunization techniques of Mishell and Dutton (J. Exp. Med. 126: 423, 1967), which have been highly successful in mouse culture systems.

4) LDA in a Chicken Model System. To further identify the nature of lymphocytes which cooperate with LDA, chickens immunized with human leukocytes are being studied. This work, which is being done in cooperation with Dr. Michael Blaese, involves the same in vitro methods as noted above. The unique procedure in this system involves bursectomy (elimination of B lymphocytes), the efficiency of which Dr. Blaese monitors by serum immunoglobulin levels and cell surface immunoglobulin determinations.

5) Tumor Specific Heterologous LDA in a Rabbit-Mouse Model System. The basic postulate of this approach is that heterologous tumor specific LDA will have an antitumor effect in situ. Rabbits have been immunized with spontaneous AKR mouse leukemia cells. Antisera have been titered for LDA and complement dependent antibody by chromium release assays. Specificity has been determined by using AKR leukemia cells and normal AKR lymphoid tissues (spleen, thymus, marrow and nodes) as target cells. In addition, specificity is checked against tissues from other normal mouse strains which naturally express little or no viral antigen. Cytotoxic specificity is assessed not only by direct cytotoxicity testing but also by absorption experiments. In vivo absorption of antisera in mice is used to remove antibody activity against normal mouse tissues and

to enrich specific antitumor activity. Specificity of antisera is again checked after in vivo absorption. At this point it should be apparent if any or all of the rabbit antisera have tumor specific LDA or complement dependent antibodies. Antisera will be identified which have relatively high levels of LDA and low levels of complement dependent antibody as opposed to inverse antibody levels. Drs. Mann and Yust (Immunology Branch, DCBD, NCI) are developing methods to further separate LDA and complement dependent antibody activity by isoelectric focusing. In situ antitumor effects of heterologous antisera with high levels of lymphocyte dependent antibody will then be compared with antitumor effects of antisera having high levels of complement dependent antibody.

6) Tumor Growth Resulting From an Inadequate or Inappropriate Immune Response in a Mouse Model System. Two types of tumors are being investigated in this approach: Moloney sarcoma virus induced tumors in BALB/c mice, and methylchloranthrene chemically induced sarcomas in C57Bl/6 mice. Both tumors are known to be immunogenic and each tumor, at appropriate doses in appropriate hosts, will grow progressively. Various parameters of the immune response will be monitored in hosts with progressively growing ultimately fatal tumors. Hosts will bear either primary tumors or tumors recently adapted to in vivo passage. Immune parameters to be assessed are a) direct, cell-mediated cytotoxicity, b) lymphocyte dependent antibody, c) blocking antibodies, d) complement dependent antibody, e) memory for direct, cell-mediated cytotoxicity, and f) cytotoxic macrophages. Target cells will consist of freshly explanted tumors which are mechanically separated and enriched on a discontinuous bovine serum albumin density gradient (J. Exp. Med. 128: 681, 1968). We feel this source of target cells will best represent antigenicity and destructability of tumor as it exists in situ. An inappropriate immune response is defined here as the absence of cytotoxic lymphocytes, cytotoxic macrophages, or lymphocyte dependent antibody; blocking antibody or memory for direct, cell-mediated cytotoxicity are present. An insufficient immune response is defined here by the presence of cytotoxic cells, lymphocyte dependent antibody or macrophages and by the lack of blocking factors.

Major Findings: 1) In Vitro Immunization of Human Lymphoid Cells. Human cytotoxic lymphoid cells have been produced in vitro by sensitizing normal human peripheral blood lymphocytes against allogeneic lymphocytes using the technique of Miggianno, et al. The immune response has been increased by modifying the procedure in the following ways: immunizing in petri dishes which are rocked (Mishell and Dutton), presumably increasing cell-cell interaction; feeding cultures with AB positive human plasma and a concentrated nutrient mixture which presumably improves cell function; and addition of beta-mercaptoethanol, a reducing agent, which presumably provides one of the functions of macrophages.

2) Human Lymphocyte Dependent Antibody. Impressions of the nature of human lymphocyte dependent antibody and normal lymphocytes which it utilizes for target cell destruction have been strengthened. Thus the presence of IgG antigenic determinants on LDA has been verified. In addition, it is clear that for target cell destruction LDA can utilize lymphocytes from agammaglobulinemic patients--lymphocytes which in three cases lacked detectable surface

immunoglobulin. This observation is important because of data in animal model systems which associated LDA with thymus independent B cells. In the human, mature B cells are not necessary for LDA to be cytotoxic.

3) Lymphocytes Utilized by LDA. In order to clarify the nature of normal lymphocytes which will cooperate with LDA for target cell destruction, bursectomized chickens are being studied in cooperation with Dr. Michael Blaese. Chicken antihuman antisera have lymphocyte dependent antibody which in the presence of normal chicken spleen cells will destroy human lymphocytes. Thus the effect of bursectomy, which eliminates the B cell series, will be particularly informative regarding the nature of cells utilized by LDA.

4) Heterologous Antitumor Lymphocyte Dependent Antibody. Observations during the past year established: a) anergic cancer patients have lymphocytes that can be utilized by LDA for target cell destruction, and b) that heterologous antisera (burro and rabbit) against human tissue contain LDA which can utilize human lymphocytes for destruction of human target cells. Thus it was of interest to determine in an animal model system how effective heterologous antitumor LDA would be in controlling tumor growth in situ. This study, initiated last year, has shown that rabbits produce heterologous antibody against spontaneous AKR mouse leukemia cells and that this antibody destroys AKR leukemia cells in vitro in the presence of normal mouse spleen cells, e.g., as LDA activity. Removal of antibody activity against normal mouse tissues, specificity studies, and in vivo antitumor studies are in progress.

5) Immune Responses Against Progressively Growing Tumors. Preliminary studies during the last 2 years have suggested that peripheral blood lymphocytes from tumor bearing patients are rarely cytotoxic for freshly explanted tumor cells in 4-hour chromium release assays. Thus we established an animal model system in which this observation could be explored under more controlled circumstances. This work, which was initiated last year with MSV and MCA induced mouse tumors, has shown that single cell suspensions of viable cells can be mechanically prepared and physically enriched for tumor cells by discontinuous bovine serum albumin density gradient purification. So far identification of tumor rich gradient bands has been morphological; however, tumor transplantation studies are in progress. Lysis of these freshly explanted tumor cells by allogeneic immune cells can be detected with 4-hour chromium release assays. Attempts to detect immune effector elements in tumor bearing animals (syngeneic) are now in progress (see above under Methods Employed).

Significance to Biomedical Research and the Program of the Institute:

1) In Vitro Immunization. Recent studies have identified several host factors which will lead to a suboptimal cellular immune response against tumor antigens in situ, e.g., a) suboptimal ratios of immunizing tumor antigens and immunocompetent cells, b) active development of suppressor T cells or humoral blocking antibody, and c) "buried" tumor antigens on tumor cells. This list is growing as further observations are made on host immune responsiveness. These detrimental influences can to a large extent be bypassed in vitro, and hopefully strong immune responses against tumor antigens will result. Such results would encourage not only the study of immune lymphocytes specifically cytotoxic

for human tumor cells, but also could lead to effective, passive immunotherapy. Recent studies suggest that sensitized lymphocytes (T cells) inhibit tumors not only by direct destruction but also by releasing antitumor amplification factors. Among these are factors which draw normal host lymphoid cells into the reaction site (leukotactic factors).

2) Lymphocyte Dependent Antibody (LDA). LDA provides a mechanism by which normal lymphoid cells can be utilized for target cell destruction. Blocking activity has not been associated with LDA, e.g., if present with lymphocytes it will be cytotoxic. This is important for tumor rejection since many of the host lymphoid cells attracted to the reaction site may be nonsensitized cells. Preparation of heterologous LDA against tumor associated antigens appears attractive since it can utilize normal human lymphocytes for destruction of human target cells. Of interest is that "anergic" cancer patients have lymphocytes which can cooperate with LDA. Therapeutic trials with heterologous LDA against mouse tumors will provide a valuable framework for thinking of similar approaches with humans.

3) Immunologic Monitoring of Hosts with Progressively Growing Tumor. A uniquely significant aspect of this work is the use of freshly explanted, purified tumor cells as target cells in rapid chromium release assays. This approach minimizes deviation of either attacking cells or tumor cells from their behavior in situ. In our opinion previous studies utilizing 2-3 day in vitro assays demonstrate sensitization of the host but not the existence of cytotoxic immune cells in situ. For example, cytotoxic precursor cells, whose further maturation is blocked in an inhibitory host environment, may differentiate into cytotoxic cells during the in vitro tests. In addition, target cells which either are tissue culture lines of tumor cells or have been passaged in vivo for many generations may not be similar in antigenicity or destructability to tumor cells as they exist in situ in the primary host.

Proposed Course of Project: Portions of this proposal were planned for last year but were postponed in order to expand our effort in animal model systems.

Human. 1) Manipulate in vitro immunization of human peripheral blood lymphocytes against allogeneic transplantation antigens and autochthonous tumor antigens. 2) In collaboration with Dr. John Potter, a) study lymphocyte dependent antibody in breast cancer patients before and after tumor resection, and b) study susceptibility of freshly explanted purified human tumors to lysis in 4-hour chromium release assays, using xenogeneic immune cells. 3) Continue the study of lymphocyte dependent antibody particularly with lymphocytes from anergic cancer patients.

Animal Models. 1) Pursue development of heterologous LDA against mouse tumor cells and test its therapeutic use in situ. 2) Pursue the study of lymphocytes utilized by LDA in the bursectomized chicken. 3) Assess immune responses against progressively growing MCA or MSV induced mouse tumors.

None of these proposals represent deviation from projects underway last year except those planned in collaboration with Dr. John Potter of Georgetown University (see above).

Honors and Awards: None

Publications:

Wunderlich, J., Martin, W. J., and Macdonald, J. S.: Functional efficiency of antitumor cytotoxic lymphoid cells. In "Immunological Parameters of Host-Tumor Relationships," (Ed. D. W. Weiss), in press.

Sterioff, S., Wunderlich, J., and Williams, G.: ^{51}Cr cytotoxicity cross-matched monitoring cellular and humoral immunity in renal transplantation. Surgical Forum, p. 267, 1972.

Blaese, M., Rosenberg, E., and Wunderlich, J.: Evidence of two functionally distinct lymphocyte populations in man. In "Microenvironmental Aspects of Immunity," (Ed. B. Jankovic and K. Isakovic), Plenum, N. Y., P. 315.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5016(c)
Title: Cellular Immunity II

Principal Investigator: John R. Wunderlich, M.D., Dean L. Mann, M.D.
Section:

Lab/Branch: Immunology Branch

Goal: Study development and function of cell-mediated immune cytotoxicity in humans. Initiate relevant animal model systems.

Approach: Two independent arms of human, cell-mediated cytotoxicity have been monitored in vitro. Sensitized human peripheral blood lymphocytes which exhibit direct cytotoxicity have been detected by 4 hr. ^{51}Cr release from target cells. Cytotoxic humoral antibody which requires lymphocytes also has been detected by ^{51}Cr release from target cells. Development of cytotoxic cells has been studied by immunizing normal human lymphocytes, in vitro, against allogeneic lymphocytes. Mouse model systems have been established to study effectiveness of heterologous antisera against leukemia and to study the nature of antitumor immunity in hosts with progressively growing lethal tumors.

Progress: Human peripheral blood lymphocytes have been immunized in vitro using the Miggiano procedure. Adaption of Mishell-Dutton techniques has improved the response. Human lymphocyte dependent antibody and cytotoxic cells have been further characterized.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Allow detection of and further our understanding of specific arms of the human cellular immune response. Allow in vitro bypass of cellular immunity which is inhibited in situ.

Level of Effort: Professional Man-Years: 1/2

Serial No. NCI-5017 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of the Biological Significance of Cell Surface
Antigens with Emphasis on Histocompatibility Antigens

Previous Serial Number: Same

Principal Investigator: G. Nicholas Rogentine, Jr., M. D.

Other Investigators: Lloyd K. Everson, M.D., Donald Buell, M.D.

Cooperating Units: Ronald Yankee, M.D., Hematology and Supportive Care Branch,
NCI, Robert Trapani, Ph.D., Microbiological Associates,
Inc., Carolyn Lingeman, M.D., Carcinogenesis Branch, NCI,
Joseph Fraumeni, M.D., Epidemiology Branch, NCI,
Paul Chretien, M.D., Surgery Branch, NCI

Man Years:

Total: 2 1/8
Professional: 1 1/8
Others: 1

Project Description:

Objectives: The overall orientation of this project is to study the natural biological significance of cell surface antigens especially those of the major histocompatibility antigen system. It is known that these antigens are important in allografting, however, the emphasis of this project is to study their significance apart from transplantation.

One objective is to find associations between certain HL-A antigens and human diseases. Another objective is to study qualitative and quantitative changes of these and other antigens in normal and neoplastic cells in vivo and in vitro.

Methods Employed: Antigens on cell surfaces are assayed by standard ⁵¹Cr release or dye exclusion cytotoxicity tests. Estimates of antigen content of different cell populations are achieved by quantitative absorption of antisera and retesting the absorbed antisera against a standard target cell. HL-A typing of the lymphocytes of patients with a variety of diseases is being conducted under contract at Microbiological Associates, Inc., under the direction of Dr. Robert J. Trapani.

Human lymphoid cells are maintained in long term tissue culture in the Branch. Techniques for obtaining synchronous populations of these cells have been developed so that the antigen content of a cell as a function of cell cycle phase may be studied.

Major Findings: In the past year we have concentrated on studies of HL-A antigens in certain malignant diseases and in diseases which have important immunological components.

HL-A2 association was found in about 2/3 of patients suffering from acute lymphocytic leukemia (ALL). This is a significant increase. It was further found that ALL patients bearing antigen HL-A2 seem to have a longer survival. This may not only be of prognostic importance, but may eventually be of therapeutic importance, e.g., allowing for a more rational selection of chemotherapeutic versus immunotherapeutic regimens.

We have also completed a study of HL-A antigens in 103 Hodgkin's disease patients which confirms findings by others that HL-A5 is elevated in this disease, but in addition showed that this elevation was found in the mixed cellularity and lymphocyte predominance histologic types and not in nodular sclerosis. We are currently investigating HL-A antigens in patients with sarcomas, malignant melanomas, squamous cell carcinomas and adenocarcinomas on the Surgery Branch, NCI, in collaboration with Dr. Paul Chretien. These patients are of extreme interest in that they have been carefully studied for their humoral and cellular immunological responses; we hope to correlate these with HL-A type. This study is currently in progress. We have typed over 300 such patients to date. We also are typing patients with ovarian cancer (with Dr. Carolyn Lingeman) and families in which there is a high incidence of cancer (with Dr. Joseph Fraumeni). These smaller studies are in progress.

Several non-malignant disease categories have been examined. Most of these diseases demonstrate some abnormality of the immune system. These included gluten-sensitive enteropathy, dermatitis herpetiformis, multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, Sjögrens syndrome, pemphigus vulgaris, bullous pemphigoid, and leprosy. To date the studies of gluten-sensitive enteropathy and dermatitis herpetiformis, done in collaboration with Dr. W. Strober, NCI, and others have resulted in publications demonstrating that 58-88% of such patients have HL-A8, compared to the normal incidence of 22%. This is the highest incidence of an HL-A antigen in any disease state.

A physical method of obtaining unperturbed cell cycle phase synchronized human tissue culture lymphoblasts has been developed. With this method we have demonstrated a significant increase in HL-A antigen content per cell in S and G₂ phase cells as compared to G₁ cells.

We have studied an antigen on human lymphocytes which is revealed by neuraminidase treatment and which reacts with normal human sera, even autologous serum. The antigen is present on peripheral blood lymphocytes, lymphoblasts in long term tissue culture and on human red cells. Inhibition studies indicate that the antibody is directed against a carbohydrate antigen, the terminal sugar of which is D galactose.

Significance to Biomedical Research and the Program of the Institute: Knowledge of cell surface structures is of central importance in understanding immune phenomena, normal growth and differentiation and malignant transformation. Cell surface antigens are easily identified markers for surface structures. The cell surface histocompatibility antigens are a product of an extremely polymorphic genetic system which must have important survival value for the individual quite irrespective of their role in provoking allograft rejection. Investigations into the nature of these important antigens in normal and malignant cells, in different organ systems, in different phases of a cell cycle, and in different stages of cellular maturation will give important information which may help us to understand the basic processes of growth and differentiation, malignant transformation and immunological recognition.

The study of the associations of certain diseases with certain HL-A antigens has a two-fold significance if such associations are found. It will allow the identification of those members in the population who are at a higher risk and thereby allow early diagnosis or by modifying environmental factors possibly prevent the disease. The second significance lies in pointing a way to design experiments that probe the basic pathogenesis of the disease.

Neuraminidase treated tumors are being widely used in human and animal tumor immunotherapy. An understanding of the immunological nature of this enzymatic change is essential to the rational application of this promising mode of therapy. Moreover, the study of the natural, even auto-, antibodies to this neoantigen may be of great importance in understanding natural cell death or mechanisms of immune surveillance.

Proposed Course of Project: A variety of patients with diseases, especially malignancies and disorders of immunity will be typed for HL-A antigens. Correlations will be sought and experiments designed to probe the pathogenesis of the disease will be attempted.

HL-A antigens and other cell surface structures will be studied on human lymphoblasts growing in long term tissue culture. They will be studied during different phases of the cell cycle and under differing environmental conditions. When possible, comparisons of HL-A antigen content on normal and neoplastic cells will be made.

Further studies of the neoantigen revealed by neuraminidase treatment of human lymphocytes will focus on the nature of the antibody in normal human serum and on the biological significance of this phenomenon. The quantity of this antigen in the various phases of the cell cycle will be studied also.

Honors and Awards: None

Publications:

Rosenberg, S. A., Plocinik, B. A., and Rogentine, G. N., Jr.: "Unmasking" of human lymphoid cell heteroantigens by neuraminidase treatment. J. Nat. Cancer Inst., 48: 1271-1276, 1972.

Rosenberg, S. A., Rogentine, G. N.: Natural human antibodies to "hidden" membrane components. Nature New Biology 239: 203-204, 1972.

Everson, L. K., Buell, D. N., Rogentine, G. N., Jr.: Separation of human lymphoid cells into G₁, S, and G₂ cell cycle populations by use of a velocity sedimentation technique. J. Exp. Med. 137: 343-358, 1973.

Rogentine, G. N., Jr., Yankee, R. A., Gart, J. J., Nam, J., and Trapani, R. J.: HL-A antigens and disease: Acute lymphocytic leukemia. J. Clin. Invest. 51: 2420-2428, 1972.

Falchuk, Z. M., Rogentine, G. N., Jr., and Strober, W.: Predominance of histocompatibility antigen HL-A8 in patients with gluten-sensitive enteropathy. J. Clin. Invest. 51: 1602-1605, 1972.

Katz, S. I., Falchuk, Z. M., Dahl, M. V., Rogentine, G. N., Strober, W.: HL-A8: A genetic link between dermatitis herpetiformis and gluten-sensitive enteropathy. J. Clin. Invest. 51: 2977, 1972.

Graff, K. S., Simon, R. M., Yankee, R. A., DeVita, V. T., and Rogentine, G. N.: HL-A antigens in Hodgkin's disease. Submitted for publication.

Rogentine, G. N., Trapani, R.-J., Yankee, R. A., and Henderson, E. S.: HL-A antigens and acute lymphocytic leukemia. The nature of the HL-A2 association. Submitted for publication.

Whisnant, J. K., Rogentine, G. N., Jr., and Robbins, J. B.: Studies on mechanism of susceptibility to H. influenzae type b disease: Relationship of erythrocyte and histocompatibility antigens with H. influenzae type b meningitis and epiglottitis. In Symposium on H. influenzae Type b Disease, Vanderbilt University Press, in press.

Amos, B., et al., The biological significance of histocompatibility antigens. Fed. Proc. 31: 1087-1104, 1972.

Rosenberg, S. A., and Einstein, A. B.: Sialic acids on the plasma membranes of cultured human lymphoid cells. J. Cell. Biol. 53: 466-473, 1972.

Rosenberg, S. A., and Rogentine, G. N., Jr.: Natural antibodies in human immune surveillance Surg. Forum XXIII: 96-98, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5017 (c)

Title: Studies of the biological significance of cell surface antigens with emphasis on histocompatibility antigens.

Principal Investigator: G. N. Roentine, Jr., M.D., and L. K. Everson, M.D.
Section:

Lab/Branch: Immunology Branch, DCBD, NCI

Goal: To obtain information about the natural biological significance of cell surface antigens, especially those of the major histocompatibility antigen system. To correlate various disease states with HL-A antigens.

Approach: HL-A and other antigens are assayed by ^{51}Cr or eosin dye exclusion cytotoxicity tests on normal and neoplastic cells. Quantitative absorptions are conducted to assess quantity of antigen.

Tissue culture cells are fractionated into cell cycle specific phases by a Ficoll density gradient technique. HL-A and other antigen content is measured on these cells.

Lymphoid cells are treated with neuraminidase in some experiments.

Progress: Several disease-HL-A associations have been demonstrated: HL-A2 and acute lymphocytic leukemia, HL-A8 with sprue and dermatitis herpetiformis, HL-A5 with Hodgkin's disease.

HL-A antigen content on a per cell basis was increased in S and G₂ cell cycle phases as compared with G₁. The cells studied were human lymphoblasts in tissue culture.

Neuraminidase treatment of human lymphocytes reveals an antigen reactive with normal human serum, even autologous serum. This antibody can be inhibited by simple sugars.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Knowledge of cell surface structures is of central importance in understanding normal growth and differentiation as well as malignant transformation. The HL-A cell surface antigenic marker is ideal for studying cell surface modifications.

Level of Effort: Professional Man-Years: 1 1/8

Serial No. NCI-5018 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Characterization of Cell Surface Molecules on Lymphoid Cells and Tumor Cells

Previous Serial Number: NCI-5018 (c)

Principal Investigator: Pierre A. Henkart, Ph. D.

Other Investigators: Richard I. Fisher, M. D. and William D. Terry, M. D.

Cooperating Units: Charles W. Todd, Ph. D., Duarte, California

Man Years:

Total: 3 1/8
Professional: 2 1/8
Other: 1

Project Description:

Objectives: 1) To identify and characterize chemically several important molecules of the lymphocyte cell surface in man and the mouse. These include the glycoproteins which are the surface receptors for the mitogens Concanavalin A and phytohemagglutinin, as well as other biologically important surface molecules. 2) To characterize chemically the carcinoembryonic antigen of human colon cancer.

Methods Employed: 1) Cell surface proteins were labeled by the technique of iodination with radioactive iodine catalyzed by lactoperoxidase. Solubilization of the cell membrane was carried out with nonionic detergent, followed by addition of the mitogen to bind its receptor; the receptor-mitogen complex was precipitated by specific antimitogen antibody and washed. These precipitates were dissolved with SDS and run in SDS gel electrophoresis. 2) Purified carcinoembryonic antigen was fractionated by ion exchange chromatography and isoelectric focusing. The various fractions were analyzed by the CEA radioimmunoassay, sialic acid, amino acid, and amino sugar analysis, and by Ouchterlony gel diffusion using anti-CEA and lectins.

Major Findings: 1) Human peripheral lymphocyte surface receptors to both PHA and Con A are detected by our technique; binding is specifically inhibited by sugar haptens and glycoproteins known to bind to the mitogens.

On SDS gels the PHA binding material gives two major peaks in the molecular weight range of 40-60,000 with minor amounts of other material. Con A binding labeled material gives two large peaks in the same positions as PHA plus a third peak and a greater amount of specifically inhibitable background label attributed to other Con A binding glycoproteins. The same two mitogen receptors appear to be present in at least one of the cultured human lymphoid cell lines. Mouse spleen cells appear to have at least one similar mitogen receptor.

2) Purified CEA is heterogeneous with respect to charge, as shown by 4-5 peaks on ECTEOLA cellulose and 7-12 peaks on isoelectric focusing. Amino acid analysis showed similar (but not precisely identical) protein composition in the various peaks, while sialic acid content varied directly with the acidity of the separated fraction. This led to the hypothesis that variations in sialic acid were responsible for the heterogeneity. Neuraminidase digestions which removed 97% of the sialic acid were performed on CEA preparations, followed by isoelectric focusing. Neuraminidase digested CEA was as heterodisperse in these experiments as was undigested CEA, although the equilibrium pH's were less acidic. Thus, sialic acid is not solely responsible for CEA's charge heterogeneity.

Significance to Biomedical Research and the Program of the Institute:

1) Basic general knowledge of cell surface molecules of higher organisms is still rudimentary. At present it is unclear how many different molecules are exposed to the exterior and how they mediate their many functions of recognizing external molecules and other cells. Progress is being made using red blood cells but it is not clear how similar these cells are to other types. I have chosen to study lymphoid cells because they are probably more typical of other cells in the body, they have many functional cell surface molecules, and because they can be grown in large quantities in culture.

2) Basic knowledge of the chemistry of cell surface molecules is needed in order to manipulate them in a rational way to obtain clinically beneficial results. In the case of tumor antigens, perhaps including CEA, immunity directed against these antigens must be stimulated. The availability of pure and chemically defined tumor antigens may allow patients to be immunized against their own tumors.

Proposed Course of Project: T and B lymphocytes will be prepared and the presence of mitogen receptors in each assayed. Antibody to pokeweed mitogen is being prepared so its receptor can be isolated by our technique. Other methods of solubilization of the receptor will be investigated (enzymatic digestion, "shedding" and hypotonic shock). Several experiments are planned to answer the question of whether or not the receptors we are detecting are the functional ones in mitogenesis.

Other lymphocyte receptors will also be analyzed by this technique. At present we are beginning to look at surface immunoglobulin on human lymphocytes.

Honors and Awards: None

Publications:

Terry, W. D., Henkart, P. A., Coligan, J. E., and Todd, C. W.: Structural studies of the major glycoprotein in preparation with carcinoembryonic antigen activity. J. Exp. Med. 136: 200-204, 1972.

Coligan, J. E., Henkart, P. A., Todd, C. W., and Terry, W. D.: The electrophoretic heterogeneity of CEA. Immunochemistry, in press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5018 (c)

Title: Characterization of Cell Surface Molecule on Lymphoid Cells and Tumor Cells

Principal Investigator: Pierre A. Henkart, Ph. D.

Section:

Lab/Branch: Immunology Branch

Goal: 1) To identify and characterize the glycoprotein lymphocyte surface receptors which bind mitogens. 2) To characterize chemically the carcino-embryonic antigen of human colon cancer.

Approach: 1) Cell surface proteins were labeled by the technique of iodination with radioactive iodine catalyzed by lactoperoxidase. Solubilization of the cell membrane was carried out with nonionic detergent, followed by addition of the mitogen to bind its receptor; the receptor-mitogen complex was precipitated by specific antimitogen antibody and washed. These precipitates were dissolved with SDS and run in SDS gel electrophoresis. 2) Purified carcinoembryonic antigen was fractionated by ion exchange chromatographic and isoelectric focusing. The various fractions were analyzed by the CEA radioimmunoassay, sialic acid, amino acid, and amino sugar analysis, and by Ouchterlony gel diffusion using anti-CEA and lectins.

Progress: 1) Human peripheral lymphocyte surface receptors to PHA and Con A are detected by our sandwich precipitation technique; binding is specifically inhibited by sugar haptens. On SDS gels the PHA binding material gives two peaks in the molecular weight range of $40-60 \times 10^3$. Con A binding material gives two major peaks at the same position, with additional minor peaks. 2) Purified CEA is heterogeneous with respect to charge. Complete neuraminidase digestion does not decrease this heterogeneity, although the antigen molecules become less acidic.

Significance for Cancer Research (NCP Objective 3 Approach 5)

These studies provide basic information on the structure and function of the cell membrane and the molecules associated with the cell surface of tumor cells. A more complete knowledge of such cell surface structures is needed to understand how tumor cells differ from normal cells, and how these differences can be exploited in therapy and diagnosis of cancer.

Level of Effort: Professional Man-Years: 2 1/8

Serial No. NCI-5019 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Structural and Immunochemical Analysis of Membranes of
Normal and Malignant Cells and of Products of These Cells

Previous Serial Number: Same

Principal Investigator: William D. Terry, M. D.

Other Investigators: Howard B. Dickler, M. D., Richard W. Smith, M. D.

Cooperating Units: Vincent Marchesi, M. D., NIAMD; Erkki Ruoshlati, M. D.,
Helsinki, Finland

Man Years:

Total: 1 3/4
Professional: 3/4
Others: 1

Project Description:

Objectives: Attempts will be made to isolate and characterize membrane proteins which 1) appear to be associated with the malignant state, and/or 2) appear to be important in acquiring an understanding of membrane structure, and 3) appear to have functional significance in the immune response. Attempts will also be made to develop reagents directed against differentiation antigens.

Methods Employed: A variety of procedures are used to solubilize membranes, followed by isolation of protein components by gel filtration, column chromatography, isoelectric focusing, and other forms of preparative electrophoresis. Isolated proteins are assessed for purity by acrylamide gel electrophoresis, and immunochemical techniques. Amino acid compositions of proteins and enzymatically prepared fragments are determined on the amino acid analyzer. Sequence analysis is carried out with manual and automatic sequencing procedures. Immunochemical studies utilize complement dependent cytotoxicity assays.

Major Findings: 1) A red cell membrane glycoprotein has been partially sequenced. The overall pattern found was one in which one end of the molecule contained all of the carbohydrate residues and an amino acid sequence of

unremarkable nature. Two-thirds of the way to the carboxy-terminus of this molecule, a hydrophobic stretch of about 30 residues was identified. Possible polymorphism relating to the M and N antigens has been identified.

2) An antiserum has been prepared against human fetal thymocytes and absorbed with long term lymphoid cell lines which have immunoglobulin on their membranes and are therefore considered to be analogous to B cells. The absorbed antiserum has relative specificity for thymus cells, as opposed to "B" cells, in a cytotoxicity assay, but lacks specificity by fluorescence.

3) Partial amino acid sequences of cyanogen bromide fragments of human α -fetoprotein have been determined. It is clear that this protein is not homologous with albumin.

4) The interaction of heat aggregated human immunoglobulin and lymphocytes is being studied using fluorescent techniques, in order to characterize the receptor for such complexes. Such studies have revealed this receptor to be present on "B" lymphocytes and binding is not dependent on complement, temperature, pH, or divalent cations. The receptor is distinct from surface Ig, appears to be specific for the Fc portion of altered or complexed immunoglobulin, and is a protein or glycoprotein. It is a useful marker for studying lymphocyte populations in disease states (e.g. CLL) as it can detect B lymphocytes which do not bear surface Ig.

Significance to Biomedical Research and the Program of the Institute:

Characterization of tumor associated antigens is of major importance in determining the relationship of these structures to normal cellular membrane components.

The structural information derived from the red cell membrane glycoprotein has provided for the first time experimental evidence supporting the theoretical model for membrane structure that has been proposed on the basis of indirect evidence.

The availability of reagents capable of distinguishing various populations of human lymphoid cells will help in assessing the role of these populations in host responses to tumors.

Proposed Course of Project: Studies of the structure of these membrane proteins will be continued and an attempt will be made to obtain complete sequences for both proteins. Additional characterization of the antithymus cell antiserum will be undertaken. Further studies on the structure of α -fetoprotein will be performed. The nature and significance of the aggregated γ G receptor on B cells will be studied further.

Honors and Awards: None

Publications:

Segrest, J. P., Jackson, R. L., Marchesi, V. T., Guyer, R. B., and Terry, W. D.: Red cell membrane glycoprotein: Amino acid sequence of an intramembranous region. Biochem. Biophys. Res. Comm. 49: 964-969, 1972.

Smith, R. W., Terry, W. D., Buell, D. N., and Sell, K. W.: An antigenic marker for human thymic lymphocytes. J. Immunol. 110: 884-887, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5019 (c)

Title: Structural and Immunochemical Analysis of Membranes of Normal and Malignant Cells and of Products of These Cells

Principal Investigator: William D. Terry, M. D.

Section:

Lab/Branch: Immunology Branch

Goal: To isolate and characterize membrane structures which 1) appear to be associated with the malignant state and 2) appear to be important in membrane function and structure.

Approach: 1) Membranes are solubilized and proteins are isolated and characterized. 2) Membrane receptors are studied and characterized using immunofluorescent techniques. 3) Membrane antigens are identified by cytotoxic assays. 4) Soluble products, associated with the malignant state, are purified, and chemically characterized.

Progress: 1) The major red cell membrane glycoprotein has been partially sequenced. 2) An antiserum with specificity for human thymic related cells has been prepared. 3) The partial amino acid sequence of human α -fetoprotein has been obtained. 4) A receptor for aggregated γ G has been associated with the surface of B cells and shown to be a dependable marker for this type of cell.

Significance for Cancer Research (NCP Objective 3 Approach 5)

1) Knowledge of the structure and function of the cell surfaces of malignant cells and immunocompetent cells is important in understanding the malignant process, as well as the function of lymphoid cells. 2) Knowledge of the structure of carcinofoetal proteins should aid our understanding of the relationship of embryonic and malignant processes.

Level of Effort: Professional Man-Years: 3/4

Serial No. NCI-5020 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunoprophylaxis and Immunotherapy of Tumors in Experimental Animals

Previous Serial Number: None

Principal Investigator: William D. Terry, M.D.

Other Investigators: Lloyd Everson, M.D.

Cooperating Units: David Lavrin, Ph.D., Contract NIH-70-2207 with Litton Bionetics, Inc.; Joseph A. Belladonna, M.D., Radiation Branch, NCI

Man Years:

Total: 1/4
Professional: 1/4
Others: 0

Project Description:

Objectives: To establish systems and test various approaches to the immunotherapy of tumors in experimental animals.

Methods Employed: Tumors are induced in mice by a variety of methods, inducing injection of methylcholanthrene. Tumors are harvested and frozen for subsequent use. Animals are immunized by conventional techniques. Additional tumors are treated with neuraminidase and increased immunogenicity tested.

Major Findings: Multiple sera containing "deblocking factor" were studied for their ability to favorably alter the course of established MCA induced tumors. No consistent effect was detected.

Significance to Biomedical Research and the Program of the Institute: BCG is being used in human immunotherapy, and it is important in both prophylaxis and therapy models to determine the possible beneficial and deleterious effects of this nonspecific stimulator of the immune system.

Proposed Course of Project: A new series of studies using neuraminidase treated tumor cells in the immunotherapy of animal tumors has been initiated. Immunotherapy studies will be extended to humans in a collaborative project with the Surgery Branch, NCI.

Honors and Awards: None

Publications:

Lavrin, D. H., Rosenberg, S. A., Connor, R. J., and Terry, W. D.: Immuno-
prophylaxis of methylcholanthrene-induced tumors in mice using Bacillus
calmette-guerin (BCG) and methanol residue (MER). Cancer Res. 33: 472-
477, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5020(c)

Title: Immunoprophylaxis and Immunotherapy of Tumors in Experimental Animals

Principal Investigator: William D. Terry, M.D.

Section:

Lab/Branch: Immunology Branch

Goal: To establish systems and test various approaches to the immunotherapy of tumors in experimental animals.

Approach

- 1) BCG is used as an immunostimulant in tumor-bearing animals.
- 2) Deblocking factors are tested for effectiveness of therapy in tumor-bearing animals.
- 3) Neuraminidase treated cells, both fresh and cultured, are tested for their ability to cause regression of established tumors in mice.

Progress:

- 1) Extensive studies of deblocking factors in mice with MCA induced tumors showed no consistent significant effects.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Approaches whereby autologous or allogeneic tumors of increased immunogenicity can cause regression of established tumors are applicable to the therapy of human cancer.

Level of Effort: Professional Man-Years: 1/4

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Idiotypes of Antibodies to Defined Histocompatibility Antigens

Previous Serial Number: None

Principal Investigator: David H. Sachs, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1
Professional: 1/4
Others: 3/4

Project Description:

Objectives: To produce anti-idiotypic antibodies against the presumed receptors which detect major histocompatibility antigens, and to determine the effects of these antibodies on the cellular and humoral reactions elicited by such antigens.

Methods Employed: Congenic resistant strains of mice differing only at their major histocompatibility loci have been reciprocally immunized in order to produce antibodies of known, restricted specificity. The antibodies have been characterized by assays of complement-mediated lymphocytotoxicity.

Antibodies against individual antigenic specificities have been isolated by absorption onto lymphoid cells bearing those specificities, and the antibody-coated cells have then been injected back into syngeneic animals in order to immunize against the "receptor" portions of the antibody molecules. Sera have been collected from these animals after multiple immunizations and are being characterized for the presence of antibodies capable of reacting with the appropriate antihistocompatibility antigen receptors of antibodies and cells. Tests include inhibition of cytotoxicity, binding to solid-phase immunoabsorbents, and in vitro and in vivo blocking of the immune response to these antigens.

Similar protocols are also being applied to antibodies raised in inbred rats differing at multiple histocompatibility loci, and to cross-species systems.

Major Findings: 1) One of the antisera produced in congenic resistant mice has been shown to contain an additional specificity to that expected in this strain combination. This specificity has been shown to be linked to H-2, to reside in or be closely linked to the K region of H-2, and to be present on a restricted population of lymphoid cells.

2) Multiple immunizations with coated cells were required before any inhibition of cytotoxicity by the immune serum could be demonstrated in the congenic resistant mouse combination. Noncongenic rat combinations and cross-species immunizations led to inhibitory sera more quickly. The specificity of this inhibition and the presence or absence of anti-isotype and antiallotype in the immune sera are presently being investigated.

Significance to Biomedical Research and the Program of the Institute:

1) The new, H-2-linked specificity we have found may provide a new cell-surface marker for a distinct population of lymphoid cells. Since a variety of immune response genes have also been localized to the H-2 K region, this population of cells may be of significance in the immune response to such antigens - an hypothesis which can be tested.

2) The specificity of the receptor for histocompatibility antigens should reside in the variable portion of the heavy and light chains of the relevant antibody molecules. Thus, antibodies against such receptors might be termed "anti-idiotypic" antibodies. Such antibodies have been shown by other workers (Nisonoff, et al.) to be produced upon immunization with antibodies to certain well-defined haptenic antigens. Furthermore, such anti-idiotypic antibodies have been found to affect immune responses to these antigens. Such antibodies, if obtained, could thus provide a new approach to modification of the immune response to cell-surface antigens.

Proposed Course of Project: Extensive testing as outlined above will be required in order to determine whether or not anti-idiotypic antibodies have been produced by any of our immunization protocols. If we can demonstrate the presence of such antibodies serologically, we will then attempt to purify them by selective immunoabsorption on columns bearing the appropriate immunoglobulins. The effects of these antibodies on in vitro cellular immune responses (e.g., MLC, cellular cytotoxicity) and on in vivo cellular and humoral responses to tissue grafts will then be tested.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5021(c)

Title: Idiotypes of Antibodies to Defined Histocompatibility Antigens

Principal Investigator: David H. Sachs, M.D.

Section:

Lab/Branch: Immunology Branch

Goal: 1) To produce anti-idiotypic antibodies against the presumed receptors which detect major histocompatibility antigens, 2) to determine the effects of these antibodies on the cellular and humoral reactions elicited by such antigens.

Approach: Congenic resistant strains of mice differing only at their major histocompatibility loci have been reciprocally immunized. The antibodies produced have been characterized by assays of complement-mediated lymphocytotoxicity. Antibodies against individual antigenic specificities have been isolated by absorption onto lymphoid cells bearing those specificities, and the antibody-coated cells have then been injected back into syngeneic animals in order to immunize against the "receptor" portions of the antibody molecules. Sera from these animals are being characterized for the presence of antibodies capable of reacting with the appropriate antihistocompatibility antigen receptors of antibodies and cells. Tests include inhibition of cytotoxicity, binding to solid-phase immunoabsorbents, and in vitro and in vivo blocking of the immune response to these antigens. Similar protocols are also being applied to antibodies raised in inbred rats differing at multiple histocompatibility loci, and to cross-species systems.

Progress: 1) In the process of characterizing our congenic antisera, we have discovered a new cell surface specificity. This specificity appears to be closely linked to the K region of H-2, and to be present on a restricted population of lymphoid cells. 2) Multiple immunizations with coated cells have produced sera capable of inhibition of cytotoxicity. The specificity of this inhibition and the presence or absence of anti-isotype and anti-allotype in the immune sera are presently being investigated.

Significance for Cancer Research (NCP Objective 3 Approach 5)

1) See Annual Report. 2) Anti-idiotypic antibodies to cell surface receptors may provide a new approach to modification of the immune response.

Level of Effort: Professional Man-Years: 1/4

Serial No. NCI-5022 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Genetics of the Idiotypic of a Human γ A Myeloma Protein

Previous Serial Number: None

Principal Investigator: David H. Sachs, M. D.

Other Investigators: William D. Terry, M. D. and Joseph D. Robinson, M. D.

Cooperating Units: None

Man Years:

Total: 1
Professional: 1
Others: 0

Project Description:

Objectives: 1) To determine whether or not the idiotype of human myeloma proteins, as classically defined, also includes restricted allotypes which can be detected in the normal immunoglobulin pools of related individuals. 2) To determine whether or not the idiotype of a human myeloma protein can serve as a TSTA (Tumor Specific Transplantation Antigen).

Methods Employed: A 19 year old male with recently diagnosed γ A myeloma, both of whose parents are alive and healthy, was selected for this study. Plasma cells from a bone marrow biopsy were frozen for possible future use. The patient's serum was subjected to ion exchange and Sephadex chromatography, and a relatively homogeneous preparation of his γ A myeloma protein was thus obtained. Animals were immunized with this protein, and subsequent sera were screened by Ouchterlony analysis for the presence of idiotypic reactions.

γ A containing globulin fractions were prepared from normal human serum, and from serum from the patient's mother and father. The putative anti-idiotypic antiserum was characterized by quantitative precipitation analysis with each of these fractions. Solid immunoabsorbent columns bearing each of these fractions and a column bearing the patient's myeloma protein have been prepared. Selective immunoabsorption on these columns will be used to distinguish possible idiotypes and allotypes detected by the heteroantiserum.

Major Findings: 1) Early bleedings after a single immunization with the myeloma protein yielded sera which showed spurring in Ouchterlony analysis characteristics of possible anti-idiotypic reactions. Later bleedings showed less clear-cut reactions. 2) Spurring was observed between this antiserum and the patient's serum even after treatment had caused a return of the patient's electrophoretic pattern to normal, indicating probable continued presence of the abnormal protein. 3) Precipitin analyses indicate that the patient's myeloma protein may bear a determinant shared by the father's A immunoglobulins but not by the mother's nor by pooled normal immunoglobulins.

Significance to Biomedical Research and the Program of the Institute:

1) The patient's young age and the availability of both parents as serum donors presents a unique opportunity to study the genetics of idio- type inheritance in man. Since all alloantigens of the patient must have derived from either one or the other of his parents, absorptions of anti- myeloma antisera with globulin fractions from the two parents should permit unequivocal distinction between restricted allotypes and true idio- type of the myeloma. 2) If the myeloma idio- type can be shown to also be a TSTA of the tumor cells or of the precursor lymphocytes (of the restricted allotype), then it may provide a marker for tumor specific immunotherapy.

Proposed Course of Project: Characterization of the anti- myeloma antibodies will be carried out as outlined above. If restricted allotype is identified, it will be examined in as many family members as possible, both as a humoral specificity and as a possible cell surface antigen.

If true anti-idiotypic antibodies are found, they will likewise be used to screen family members' serum and lymphoid cells. Reactions with the patient's lymphocytes and stored plasma cells will be characterized by fluorescent-labeled antibodies and by cytotoxic reactions.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5022 (c)

Title: Genetics of the Idiotype of a Human γ A Myeloma Protein

Principal Investigator: David H. Sachs, M. D.

Section:

Lab/Branch: Immunology Branch

Goal: 1) To determine whether or not the idiotype of human myeloma proteins includes restricted allotypes. 2) To determine whether or not the idiotype of a human myeloma protein can serve as a TSTA (Tumor Specific Transplantation Antigen).

Approach: A 19 year old male with recently diagnosed γ A myeloma, both of whose parents are alive and healthy, was selected for this study. Plasma cells from a bone marrow biopsy were frozen for possible future use. The patient's serum was subjected to ion exchange and Sephadex chromatography, and a relatively homogeneous preparation of his γ A myeloma protein was thus obtained. Animals were immunized with this protein, and subsequent sera were screened by Ouchterlony analysis for the presence of idiotype reactions. γ A containing globulin fractions were prepared from normal human serum, and from serum from the patient's mother and father. The putative anti-idiotypic antiserum was characterized by quantitative precipitation analysis with each of these fractions. Solid immunoabsorbent columns bearing each of these fractions and a column bearing the patient's myeloma protein are being prepared. Selective immunoabsorption on these columns will be used to distinguish possible idiotypes and allotypes detected by the heteroantisera.

Progress: Early bleedings after a single immunization with the myeloma protein yielded serum which showed possible antiidiotypic reaction. The reaction was observed with the patient's serum even after treatment had caused a return of the patient's electrophoretic pattern to normal, indicating probable continued presence of the abnormal protein. Precipitin analyses indicate that the patient's myeloma protein may bear a determinant shared by the father's A immunoglobulins but not by the mother's nor by pooled normal immunoglobulins.

Significance for Cancer Research (NCP Objective 3 Approach 5): 1) The patient's young age and the availability of both parents as serum donors presents a unique opportunity to study the genetics of idiotype inheritance in man. 2) If the myeloma idiotype can be shown to also be a TSTA of the tumor cells, it may provide a marker for tumor specific immunotherapy.

Level of Effort: Professional Man-Years: 1

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Transplantation Antigens of Swine

Previous Serial Number: None

Principal Investigator: David H. Sachs, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	3/8
Professional:	1/8
Others:	1/4

Project Description:

Objectives: To develop at least two strains of miniature swine, each homozygous for a different set of histocompatibility antigens at the major histocompatibility locus (SL-A). The animals will subsequently be used for in vivo experiments in organ and tissue transplantation and as a source of large numbers of cells from which cell surface antigens can be isolated, purified and characterized chemically.

Methods Employed: Unlike classical inbreeding schemes which require about twenty generations to produce homozygosity, the approach being used with these swine involves selective breeding on the basis of histocompatibility typing of parents and offspring.

Four males and four females were purchased from commercial sources, and mating pairs were selected to be as varied as possible in order to assure the selection of different histocompatibility genes. Skin grafts were exchanged between the members of each pair and sera were obtained from the animals two weeks after the rejection of the grafts. The sera were tested for cytotoxic antibodies by lymphocytotoxic typing, and pairs which produced strong cytotoxic antibodies were bred.

Offspring from each breeding were tested serologically to determine which histocompatibility antigens had been inherited from the parents, and offspring which could be shown to possess the same antigen combinations were selected

for further breeding.

Major Findings: 1) Naturally occurring anti-red blood cell antibodies of swine are cytotoxic to lymphocytes (unlike their human counterparts) and must be absorbed from sera with packed red cells before leukocyte typing is performed.

2) Skin grafts were rejected in 8-14 days in each pair tested, and the rejection was accompanied by the production of high-titered lymphocytotoxic antibodies in all but one pair.

3) Breeding was successful and typing of the first generation offspring showed four pairs which had inherited appropriate antigen combinations for further breeding.

Significance to Biomedical Research and the Program of the Institute: One of the major problems in the study of cell surface antigens of human beings is lack of control of genetic constitution. The use of mice and rats as experimental models avoids this problem, but creates two new ones: 1) physiologically and anatomically these animals are often so different from human beings as to make comparisons and applications of findings difficult. 2) The size of these rodents makes it extremely difficult to obtain sufficient cells and tissues to permit quantitative chemical characterization of relevant cell surface antigens.

For both of these reasons it would be desirable to have animals of size comparable to human beings, whose genetic constitution with respect to histocompatibility could be controlled. The miniature pig, which attains an adult weight of about 200 lb. seemed ideal for this purpose, and to date the experimental breeding pattern outlined above appears to be working quite well.

Proposed Course of Project: The breeding plan and typing will be continued as outlined above. Presumably one or two more generations will suffice to fix the major histocompatibility loci of at least two of the present lines. At that point the lines will be established and separated, and herds of any required size in each line can be produced.

Experiments envisioned once these lines are established include: 1) characterization of the immune response to transplantation within and between lines (in vitro and in vivo), 2) development of a transplantable tumor within one or more of the lines, and 3) isolation, purification and characterization of the major transplantation antigens of one or more of the lines.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5023(c)

Title: Transplantation Antigens of Swine

Principal Investigator: David H. Sachs, M.D.

Section:

Lab/Branch: Immunology Branch

Goal: To develop at least two strains of miniature swine, each homozygous for a different set of histocompatibility antigens at the major histocompatibility locus (SL-A).

Approach: Unlike classical inbreeding schemes which require about twenty generations to produce homozygosity, the approach being used with these swine involves selective breeding on the basis of histocompatibility typing of parents and offspring. Four males and four females were purchased from commercial sources, and mating pairs were selected to be as varied as possible in order to assure the selection of different histocompatibility genes. Skin grafts were exchanged between the members of each pair and sera were obtained from the animals two weeks after the rejection of the grafts. The sera were tested for cytotoxic antibodies by lymphocytotoxic typing, and pairs which produced strong cytotoxic antibodies were bred. Offspring from each breeding were tested serologically to determine which histocompatibility antigens had been inherited from the parents, and offspring which could be shown to possess the same antigen combinations were selected for further breeding.

Progress: Skin grafts were rejected in 8-14 days in each pair tested, and the rejection was accompanied by the production of high titered lymphocytotoxic antibodies in all but one pair. Breeding was successful, and typing of the first generation offspring showed four pairs which had inherited appropriate antigen combinations for further breeding.

Significance for Cancer Research (NCP Objective 3 Approach 5):

These pigs will provide a model animal of size comparable to human beings whose genetic constitution with respect to histocompatibility can be controlled. The animals will serve as a source of large numbers of homozygous cells from which cell surface antigens can be isolated, purified and characterized chemically.

Level of Effort: Professional Man-Years: 1/8

Serial No: NCI-5024(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Antibodies to Cell-Surface Antigens of Leukemic Cells

Previous Serial Number: None

Principal Investigator: Howard B. Dickler, M.D., David H. Sachs, M.D.

Other Investigators: William D. Terry, M.D., Joseph D. Robinson, M.D.

Cooperating Units: None

Man Years:

Total:	1
Professional:	1
Others:	0

Project Description:

Objectives: 1) To develop antisera specific for tumor-related antigens on the surfaces of human chronic lymphocytic leukemia cells. 2) To determine the nature of the antigens detected. 3) To develop similar reagents for leukemic cells of mice to serve as a model for experiments using the anti-human leukemia reagents.

Methods Employed: Human: Patients with a confirmed diagnosis of CLL and with both biologic parents alive were selected. Leukemic cells were purified from their peripheral blood and rabbits were immunized with these cells. The antisera are being tested for reactions with lymphoid cells and immunoglobulins of the patient, the patient's parents, other CLL patients, cultured lymphoblast lines, and normal controls. The intent will be to render the antisera specific for tumor-related antigens by absorption with the other cells and globulins. Tests for specificity of reactions will include cytotoxic screening, precipitin analyses, and immunofluorescent staining.

Mouse: Similar immunization and absorption procedures will be used on two or more recently derived lymphatic leukemias of mice which grow in ascites form. Normal lymphoid cells from syngeneic animals and from allogeneic animals will serve as the control cells for absorptions.

Major Findings: 1) Two appropriate patients have been selected and rabbit antisera have been raised against the leukemic cells of each. The antisera

have high cytotoxic titers against normal and cultured lymphoid cells. 2) Sera raised against one of the leukemias also show precipitin reactions with normal human globulins, and show spurring in double diffusion tests indicative of possible idiotypic reaction with a serum protein of the patient. 3) Two appropriate mouse tumors have been obtained (from Dr. B. Sanford) and are presently being passaged to standardize their growth characteristics.

Significance to Biomedical Research and the Program of the Institute:

1) If detected, a tumor-related antigen common to all CLL cells would be of immediate use in diagnostic testing. 2) A tumor-specific reaction could have applications in the realm of immunotherapy. The mouse model would be of great importance at this stage. 3) If a true idioype is detected in the surface immunoglobulin of the CLL cells, it would suggest the clonal nature of this disease. 4) The serologic reagents developed may provide an experimental approach to isolation and characterization of the relevant tumor-related antigens, which might in turn provide information on the etiology of the tumor.

Proposed Course of Project: The availability of the patient's parents provides the important opportunity to control for specificities against inherited normal cell-surface antigens. The anti-CLL antisera will be absorbed with cultured human lymphoblastoid cells to remove antibodies against the majority of species-specific antigens. The reagents will then be absorbed with parental normal lymphocytes, at which point only tumor-related specificities should persist. Alternate absorption protocols will be used to develop possible serologic specificities toward "B" cell antigens, HL-A antigens, and possible idiotypic antigens of the cell-surface immunoglobulins.

Parallel immunizations and absorptions will be carried out for the mouse leukemia model. In vivo effects of the putative tumor-specific antibodies will be studied, and localization studies will be performed.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5024(c)

Title: Antibodies to Cell Surface Antigens of Leukemic Cells

Principal Investigator: Howard B. Dickler, M.D., and David H. Sachs, M.D.
Section:

Lab/Branch: Immunology Branch

Goal: 1) To develop antisera specific for tumor-related antigens on the surfaces of human chronic lymphocytic leukemia cells. 2) To determine the nature of the antigens detected. 3) To develop similar reagents for leukemic cells of mice to serve as a model for experiments using the antihuman leukemia reagents.

Approach: Human: Patients with a confirmed diagnosis of CLL and with both biologic parents alive were selected. Leukemic cells were purified from their peripheral blood and rabbits were immunized with these cells. The antisera are being tested for reactions with lymphoid cells and immunoglobulins of the patient, the patient's parents, other CLL patients, cultured lymphoblast lines and normal controls. The intent will be to render the antisera specific for tumor related antigens by absorption with the other cells and globulins. Tests for specificity of reactions will include cytotoxic screening, precipitin analyses, and immunofluorescent staining.

Mouse: Similar immunization and absorption procedures will be used on two or more recently derived lymphatic leukemias of mice which grow in ascites form. Normal lymphoid cells from syngeneic animals and from allogeneic animals will serve as the control cells for absorptions.

Progress: 1) Two appropriate patients have been selected, and rabbit antisera have been raised against the leukemic cells for each. The antisera have high cytotoxic titers against normal and cultured lymphoid cells. 2) Sera raised against one of the leukemias also show precipitin reactions with normal human globulins, and show spurring in double diffusion tests indicative of possible idiotypic reaction with a serum protein of the patient. 3) Two appropriate mouse tumors have been obtained (from Dr. B. Sanford) and are presently being passaged to standardize their growth characteristics.

Significance for Cancer Research (NCP Objective 3 Approach 5):

1) If detected, a tumor-related antigen common to all CLL cells would be of immediate use in diagnostic testing. 2) A tumor specific reaction could have applications in the realm of immunotherapy. The mouse model would be of great importance at this stage. 3) If a true idiootype is detected in the surface immunoglobulin of the CLL cells, it would suggest the clonal nature of this disease.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-5026 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Genetic Control of Cellular Immunity to Staphylococcal
Nuclease: Antigen-induced Stimulation of Protein and DNA
Synthesis

Previous Serial Number: None

Principal Investigator: Gene M. Shearer

Other Investigators: David H. Sachs, M.D., Barry Handwerger, M.D.,
Ronald Schwartz, M.D.

Cooperating Units: None

Man Years:

Total:	7/8
Professional:	7/8
Others:	0

Project Description:

Objectives: The objectives of this study were to establish whether the genetic control of antibody response to Staphylococcal nuclease could be extended to include differential stimulation of high and low responder lymphocytes in vitro for specific DNA or protein synthesis, and if so, to determine whether the incubation of monospecific alloantisera with the lymphocytes would inhibit the DNA or protein synthesis.

Methods Employed: Lymph node cells from high and low responder mice which had been immunized with nuclease were cultured in microtiter plates with nuclease or nuclease fragments in a serum-free culture system. The cultures were pulse labeled for DNA or protein synthesis by incorporation of radioactive thymidine or leucine. Monospecific alloantisera exhibiting specificity exclusive for H-2 were prepared using congenic B10 and B10.A mice. Other alloantisera (specific for H-2 and other differences) was prepared in DBA/1 and SJL/J mice. The "blocking" of nuclease-induced lymphocyte stimulation is to be tested by incubating the cells with the antisera prior to culture with nuclease or nuclease fragments.

Major Findings: Staphylococcal nuclease specifically stimulated protein synthesis (but not DNA synthesis) in B10.A and SJL/J high responder mouse strains, without detectably elevating protein synthesis in B10 and DBA/1 low

responder mice. This contrasts with the repeated observation that significant specific protein synthesis could not be obtained for synthetic polypeptide immunogens.

Significance to Biomedical Research and the Program of the Institute:

These observations indicate that an immunological function other than antibody production is under genetic control in the mouse. This provides an opportunity to investigate the cellular aspects of immune response gene regulation at a level other than antibody production. Furthermore, it permits an analysis of the inhibition of lymphocyte stimulation by both restricted (H-2 differences only) and non-restricted (H-2 and other differences) alloantiserum in the elucidation of a possible Ir-gene product on cell surfaces.

Proposed Course of Project: Attempts will be made to inhibit the nuclease induced stimulation of high responder lymphocytes by the monospecific alloantiserum in B10-B10.A congenic example and by the less restricted alloantiserum in the non-congenic SJL/J-DBA/1 example. Similar experiments will be performed using fragments of nuclease as well as the intact immunogen.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5026 (c)

Title: Genetic Control of Cellular Immunity to Staphylococcal Nuclease:
Antigen Induced Stimulation of Protein and DNA Synthesis

Principal Investigator: Gene M. Shearer, Ph.D.

Section:

Lab/Branch: Immunology Branch

Goal: To determine if differences in in vitro cellular immune responses to staphylococcal nuclease will follow genetic patterns observed for antibody synthesis and to test whether anti-H-2 antiserum will block responses of high responder mice.

Approach: Lymph node cells from high and low responder mice which had been immunized with nuclease were cultured in microtiter plates with nuclease or nuclease fragments in a serum-free culture system. The cultures were pulse labeled for DNA or protein synthesis by incorporation of radioactive thymidine or leucine. Monospecific alloantiserum exhibiting specificity exclusive for H-2 were prepared using congenic B10 and B10.A mice. Other alloantiserum (specific for H-2 and other differences) was prepared in DBA/1 and SJL/J mice. The "blocking" of nuclease-induced lymphocyte stimulation is to be tested by incubating the cells with the antisera prior to culture with nuclease or nuclease fragments.

Progress: Staphylococcal nuclease specifically stimulated protein synthesis (but not DNA synthesis) in B10.A and SJL/J high responder mouse strains, without detectably elevating protein synthesis in B10 and DBA low responder mice.

Significance for Cancer Research (NCP Objective 3 Approach 5)

This provides an opportunity to study the cellular aspects of immune response gene regulation at a level other than antibody production and to study genetic control of cell surface properties in relation to immune potential.

Level of Effort: Professional Man-Years: 7/8

Serial No. NCI-5027 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Identification of a Genetic Complex in the Mouse Other Than H-2 That Controls Multiple Immunological Functions and Antibody Response to More Than One Immunogen.

Previous Serial Number: None

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: David H. Sachs, M.D., Eugene C. Lozner, M.D.,
Edna Mozes, Ph.D.

Cooperating Units: Department of Chemical Immunology, Weizmann Institute of Science, Rehobot, Israel

Man Years:

Total: 1
Professional: 1
Other: 0

Project Description:

Objectives: The H-2 region in the mouse and an analogous major histocompatibility region in the guinea pig have been shown to be genetic regions which control immunological responsiveness to a number of immunogens (McDevitt and Benacerraf, Advanc. Immunol. 11: 31, 1969). Nevertheless, other less obvious genetic complexes which regulate immunological functions for a number of immunogens could also exist. The Ir-3 gene is an example of a non-H-2 linked immune response gene which controls responsiveness to the synthetic polypeptide (T,G)-Pro--L. SJL/J mice are the lowest responders to (T,G)-Pro--L, whereas DBA/1 mice are the lowest responders (Mozes, et al. J. Exp. Med 130: 493, 1969). An identical pattern of responsiveness has also recently been observed for antibody responses to Staphylococcal nuclease (see separate report of Sachs and Shearer). The purpose of the present study was to establish whether a genetic region can be identified in the mouse other than H-2 which regulates multiple immunological function) and responsiveness to a variety of immunogens.

Methods Employed: The SJL/J and DBA/1 mouse strains as well as F₁, F₂ and backcrosses between these two strains are being studied with respect to their ability to respond to Staphylococcal nuclease, (T, G)-Pro--L and mitogen stimulation, as well as their ability to reject parental bone marrow cell

grafts (hybrid histocompatibility). The hybrid histocompatibility (Hh) function may be an important marker in identifying another genetic region of immune response control, since Hh-1 has been localized in the D-region of H-2. The ability of individual F₂ and backcross animals to respond to the above mentioned antigens and immunological functions are being studied for possible association of responses as well as linkage to H-2 and coat color.

The animals are being sequentially immunized with nuclease and (T,G)-Pro--L and their sera assayed for anti-(T,G)-Pro--L activity by hemagglutination and for anti-nuclease activity by a quantitative enzymatic assay (Sachs, et al. J. Immunol. 109: 1300, 1972). The experiments are being controlled for possible antigenic competition between these two immunogens. The mitogenic response potential of lymphoid cells from individual mice was tested for response to PHA, concanavalin A and lipopolysaccharide by pulse labelling the stimulated cells for elevated protein and DNA synthesis. Expression of an Hh gene was tested by injecting parental cells into irradiated F₁, F₂ and backcross animals whose responses for the above-noted immunological parameters have been previously determined.

Major Findings: The major findings made thus far are:

1) SJL/J and DBA/1 mice exhibit differential responsiveness to mitogenic stimulation with PHA, concanavalin A, and lipopolysaccharide. Spleen and lymph node cells from SJL/J and (DBA/1 x SJL/J)F₁ elicit higher responses to PHA and concanavalin A than do lymphoid cells from DBA/1 mice. Conversely, DBA/1 lymphocytes respond slightly better to mitogenic stimulation with lipopolysaccharide than do cells from SJL/J and F₁ donors.

2) (DBA/1 x SJL/J)F₁ mice reject grafts of SJL/J parental bone marrow but not those of the DBA/1 parent, indicating that hybrid resistance does indeed occur in this strain combination.

Significance to Biomedical Research and the Program of the Institute:

If evidence can be obtained indicating the existence of a genetic region for expression of immune responsiveness other than H-2, then genetic regulation of immune responsiveness would be much more complex than is presently assumed, and another genetic complex of immune response will have been identified. Such an observation would expose an additional gene complex whose function for regulation of immune processes for both the normal and neoplastic tissue recognition could be pursued. It may be significant that the SJL/J mouse strain (which expresses immune gene functions as summarized above) is also a strain which develops viral-associated leukemia (Haran-Ghera & Kotler, Bibl. Haematol. 31: 70, 1968), and that the immune response potential of infected animals is reduced (Shearer, et al. J. Immunol. 110: 736, 1973).

Proposed Course of Project: Offspring of the F₁, F₂ and backcross mating are being individually studied with respect to their ability to respond to the immunological parameters outlined above. Should an association of two or more parameters be established, attempts will be made to link these immunological functions to known murine genetic markers.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5027 (c)

Title: Identification of a Genetic Complex in the Mouse Other Than H-2 That Controls Multiple Immunological Functions and Antibody Responses to More Than One Immunogen

Principal Investigator: Gene M. Shearer, Ph.D.

Section:

Lab/Branch: Immunology Branch, DCRD, NCI

Goal: The purpose of the present study was to establish whether a genetic region can be identified in the mouse other than H-2 which regulates multiple immunological functions and responsiveness to a variety of immunogens.

Approach: The SJL/J and DBA/1 mouse strains as well as F₁, F₂ and backcrosses between these two strains are being studied with respect to their ability to respond to Staphylococcal nuclease, (T,G)-Pro--L and mitogen stimulation, as well as their ability to reject parental bone marrow cell grafts (hybrid histocompatibility). The hybrid histocompatibility (Hh) function may be an important marker in identifying another genetic region of immune response control, since Hh-1 has been localized in the D-region of H-2. The ability of individual F₂ and backcross animals to respond to the above mentioned antigens and immunological functions are being studied for possible association of responses as well as linkage to H-2 and coat color.

Progress: SJL/J and DBA.1 mice exhibit differential responsiveness to mitogenic stimulation with PHA, concanavalin A and lipopolysaccharide. Spleen and lymph node cells from SJL/J and (DBA/1 x SJL/J)F₁ elicit higher responses to PHA and concanavalin A than do lymphoid cells from DBA/1 mice. Conversely, DBA/1 lymphocytes respond slightly better to mitogenic stimulation with lipopolysaccharide than do cells from SJL/J and F₁ donors. (DBA/1 x SJL/J)F₁ mice reject grafts of SJL/J parental bone marrow but not those of the DBA/1 parent, indicating that hybrid resistance does indeed occur in this strain combination.

Significance for Cancer Research (NCP Objective 3 Approach 4)

Identification of another genetic complex (other than the major histocompatibility region) which controls immunity could lead to the localization of genes controlling immune processes for recognition of both normal and neoplastic tissues.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-5028 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Separation of Functionally Distinct Immunocompetent Cells by Fractionation over Histamine Columns.

Previous Serial Number: None

Principal Investigator: Gene M. Shearer, Ph.D.

Other Investigators: Elizabeth Simpson, Vet. M. B., Kenneth L. Melmon, M.D.
Yacob Weinstein, Ph.D.

Cooperating Units: Division of Clinical Pharmacology, University of California Medical Center, San Francisco, Calif.

Man Years:

Total: 3/8
Professional: 3/8
Others: 0

Project Description:

Objectives: It has been recently reported that fractionation of mouse spleen cells over columns of histamine-rabbit serum albumin-Sepharose results in a depletion of the number of anti-sheep erythrocyte plaque-forming cells (PFC) passing through the columns and in an elevation of the immune response potential of the same cell suspension when transferred into irradiated recipient mice (Shearer, et al., J. Exp. Med. 136: 1302, 1972). The objective of this study was to test the effects of such cell fractionation for other immunological functions, and to elucidate the cellular mechanisms involved.

Methods Employed: Histamine-rabbit serum albumin-Sepharose (HRSA-S) and rabbit serum albumin-Sepharose (RSA-S) beads were prepared by coupling histamine to RSA with ECDI, and then binding the proteins to CNBr-activated Sepharose 4B. Cells were incubated with the bead preparations (25×10^6 cells/0.6 ml of 25% W/v beads), and then non-adherent cells separated from the adherent population by passing the cell-bead mixture through a column containing a cotton plug. Cells were eluted from the HRSA-S and RSA-S columns by mechanical means. The various fractions of cells were tested for different immunological functions, e.g., in vitro and in vivo cell mediated cytotoxicity,

graft vs host reaction, and the generation of PFC using in vivo cell transfers and in vitro Mishell-Dutton cultures.

Major Findings: The major findings can be summarized as follows:

- 1) Most of the cell mediated cytotoxic activity was found in the cells retained by the HRSA-S columns, when tested in vitro. In contrast, most of the cytotoxic precursor cell activity appeared to be in the cells that did not adhere to the HRSA-S columns when the cell mediated immunity was generated in irradiated allogeneic mice.
- 2) A similar discordance of results was observed between in vivo and in vitro experiments for humoral antibody responses to SRBC. In vivo cell transfers yielded elevated PFC responses in the cell population that did not adhere to the histamine beads whereas no effect of the column could be detected when the cells were cultured in vitro.
- 3) No effect of this type of cell separation was observed on the graft versus host response tested in vivo.
- 4) Soluble histamine introduced into the cultures reduced cell mediated cytotoxicity during the early phase of immunization (days 1 and 2) but not later (days 3 and 4).

Significance to Biomedical Research and the Program of the Institute:

These results raise the possibility that certain populations of lymphocytes may express surface receptors for histamine. The fractionation procedures can be utilized for separation of cells with different immunological functions. The differential effects of testing the separated cells in vitro and in vivo raises the possibility that radio resistant cells in the host animals may modify immune responses and/or that in vitro culture systems may permit the survival of selected cell populations. This study suggests that receptors for endogenous hormones may be expressed on lymphoid cells and they may be significant in regulating immune processes.

Proposed Course of Project: The in vivo versus in vitro results will be pursued to determine if the differences observed could be due to differences in effective antigen doses or cell concentration effects. The enhancement of the in vivo PFC response will be elucidated at the cellular level to determine whether a suppressor cell is responsible and to determine what cellular event is modified by the fractionation procedure.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5028 (c)

Title: Separation of Functionally Distinct Immunocompetent Cells by Fractionation Over Histamine Columns

Principal Investigator: Gene M. Shearer, Ph.D.

Section:

Lab/Branch: Immunology Branch, DCBD, NCI

Goal: To separate lymphoid cells on histamine-coated columns and to test the immunological function of the fractionated cell populations with respect to both cell mediated and humoral immunity.

Approach: Histamine-rabbit serum albumin-Sepharose (HRSA-S) and rabbit serum albumin-Sepharose (RSA-S) beads were prepared by coupling histamine to RSA with ECDI, and then binding the proteins to CNBr-activated Sepharose 4R. Cells were incubated with the bead preparations (25×10^6 cells/0.6 ml of 25% w/v beads), and the nonadherent cells separated from the adherent population by passing the cell-bead mixture through a column containing a cotton plug. Cells were eluted from the HRSA-S and RSA-S columns by mechanical means. The various fractions of cells were tested for different immunological functions, e.g., in vitro and in vivo cell mediated cytotoxicity, graft vs host reaction, and the generation of PEC using in vivo cell transfers and in vitro Mishell-Dutton cultures.

Progress: 1) Most of the cell mediated cytotoxic activity was found in the cells retained by histamine-bead columns, when tested in vitro. In contrast, the cytotoxic precursor cell activity appeared to be in the cells that did not adhere to the HRSA-S columns when the cell mediated immunity was generated in irradiated allogeneic mice. A similar discordance of results was observed between in vivo and in vitro experiments for humoral antibody responses to SRBC. In vivo cell transfers yielded elevated PFC responses in the cell population that did not adhere to the histamine beads, whereas no effect of the column could be detected when the cells were cultured in vitro. Soluble histamine introduced into the cultures reduced cell mediated cytotoxicity during the early phase of immunization (days 1 and 2) but not later (days 3 and 4).

Significance for Cancer Research (NCP Objective 3 Approach 5)

This study suggests that membrane receptors may exist for endogenous hormones on lymphoid cells and these receptors may be significant in regulating immune processes.

Level of Effort: Professional Man-Years: 3/8

Serial No. NCI-50²⁹(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Preparation of Tumor and Tissue Specific Antisera From Human Cancers

Previous Serial Number: None

Principal Investigator: Richard W. Smith, M.D.

Other Investigators: None

Cooperating Units: Richard L. Edelson, M.D., Marvin A. Lutzner, M.D.,
Dermatology Branch, NCI;
Philip S. Schein, M.D., Medicine Branch, NCI

Man Years:

Total: 1/2
Professional: 1/4
Others: 1/4

Project Description:

Objectives: To prepare serologic reagents with specificity for human tumor antigens or tissue specific antigens present on tumors for the purpose of diagnostic and prognostic studies for identification of tumor cells in pathological specimens and in mixed culture.

Methods Employed: Standard tissue culture techniques, immunization of rabbits and mice, absorption with cells, tissues, or serum as indicated, cytotoxic and immunofluorescent assays. Lymphoblast lines from some patients are being established in order to make available genetically identical absorbing cells.

Major Findings: Cultured melanoma cells and freshly explanted nevus cells share tissue-specific cell surface antigens which are demonstrated after extensive absorptions. These antigens are not present on blood lymphocytes of persons from whom melanoma or melanocytes were obtained. Likewise they are not present on other tumor types or on skin fibroblasts.

Significance to Biomedical Research and the Program of the Institute: Reagents for tissues or tumor specific antigens will be of great value in diagnosis of tumors if they secrete antigens into blood or urine and will aid

pathological diagnosis of surgical specimens.

Proposed Course: Attempts to prepare sera specific for antigens on melanoma, breast carcinoma, ovarian carcinoma and lymphatic malignancies will be continued.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5029 (c)

Title: Preparation of Tumor and Tissue Specific Antisera from Human Cancers

Principal Investigator: Richard W. Smith, M. D.

Section:

Lab/Branch: Immunology Branch

Goal: Development of reagents to identify tumor antigen in the human.

Approach: Tumor cells are being used to immunize animals. The antisera are being suitably absorbed and tested for specificity.

Progress: Cultured melanoma cells and freshly explanted nevus cells share tissue specific cell surface antigens.

Significance for Cancer Research (NCP Objective 3 Approach 5)

These studies are an attempt to improve diagnostic testing for cancer.

Level of Effort: Professional Man-Years: 1/4

Serial No. NCI-5030(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Development of Antisera Detecting Antigens Specific for Human Thymic Derived (T) or Bone Marrow Derived (B) Human Lymphocytes

Previous Serial Number: None

Principal Investigator: Richard W. Smith, M.D.

Other Investigators: William D. Terry, M.D., Donald Buell, M.D.

Cooperating Units: Richard L. Edelson, M.D., Dermatology Branch, NCI;
James Woody, M.D., Kenneth Sell, M.D., Naval Medical
Research Institute, Bethesda, Maryland

Man Years:

Total: 3/4
Professional: 1/2
Others: 1/4

Project Description:

Objectives: To develop reliable serologic reagents for the identification or selective elimination of human T and B lymphocytes.

Methods Employed: Heterologous antisera were prepared by immunization of various animals with fresh or cultured lymphoid cells. Depending on the specificity desired, T or B lymphoid cells were used to absorb out unwanted antibody activity. Microcytotoxicity tests were used to titrate sera for complement dependent antibodies.

Major Findings: 1) Human thymic lymphocyte specific antigen can be detected on a large population of human lymphocytes. It is not present on lymphocytes bearing surface immunoglobulin or C¹ receptors.

2) Human bone marrow lymphocyte specific antigen can be detected on lymphocytes that bear surface immunoglobulin or C³ receptors as well as on some cultured lymphoid cells which have lost these markers.

3) The immunosuppressive potency of antilymphocyte serum is directly related to the content of antibody specific for T lymphocytes.

Significance to Biomedical Research and the Program of the Institute:

The availability of potent and reliable reagents detecting human lymphocyte populations will permit a wide variety of basic and clinical investigations on normal and abnormal lymphocyte function. In the future such reagents may allow more sophisticated modification of cellular immunity or antibody responses than is presently possible. The finding that immunosuppression is related to titer of specific anti-T cell antibodies will permit monitoring of activity during ALS production and purification and should overcome many difficult logistical problems in the preparation of antilymphocyte serum. This finding will now permit ALS used in clinical transplantation to be standardized with respect to immunosuppressive activity.

Proposed Course of Project: Development or identification of T and B lymphocyte cell lines, preferably from the same normal individual, for the production and absorption of potent serum to suppress T or B cell functions in patients.

Honors and Awards: None

Publications:

Smith, R. W., Terry, W. D., Buell, D. N., and Sell, K. W.: An antigenic marker for human thymic lymphocytes. J. Immunol. 110: 884, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5030 (c)

Title: Development of Antisera Detecting Antigens Specific for Human
Thymic Derived (T) or Bone Marrow Derived (B) Human Lymphocytes

Principal Investigator: Richard W. Smith, M. D.

Section:

Lab/Branch: Immunology Branch

Goal: To develop reliable serologic reagents for the identification or
selective elimination of human T and B lymphocytes.

Approach: Heterologous sera were prepared by immunization of various animals
with fresh or cultured lymphoid cells. Depending on the desired specificity,
T or B lymphoid cells were used for absorption.

Progress: Reliable cytotoxic antisera, on specific for human T cells and
the other for B cells, have been produced.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Improved identification of malignant cell types and of the lymphocyte
involved in tumor immunity.

Level of Effort: Professional Man-Years: 1/2

Serial No. NCI-5031(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Human Lymphocyte Markers in Basic Research and in Lymphoid Diseases

Previous Serial Number: None

Principal Investigator: Richard W. Smith, M.D.

Other Investigators: None

Cooperating Units: R. Michael Blaese, M.D., Metabolism Branch, NCI;
Richard L. Edelson, M.D., Dermatology Branch, NCI;
Paul Pattengale, M.D., Paul Gerber, Ph.D., Bureau
of Biologics; Brigid G. Leventhal, M.D., Hematology and
Supportive Care Branch, NCI

Man Years:

Total: 1/2
Professional: 1/4
Others: 1/4

Project Description:

Objectives: The objectives of this project include various goals in basic and clinical immunology: 1) the comparison of sensitivity and reliability of five different markers of human T or B lymphocytes, since these markers are being used to determine the type of lymphocytes participating in different types of cellular immune reactions in vitro and in vivo. 2) To study the cells responding to mitogens and to viruses. 3) To determine the type of lymphocytes participating in cellular immune reactions in vivo and in vitro. 4) To study lymphocyte type and function in various immunodeficiency syndromes as well as in malignant diseases such as lymphoma and lymphocytic leukemia.

Methods Employed: Freshly explanted and cultured cells are being tested simultaneously for T and B lymphocyte markers. Likewise normal cord blood lymphocytes are being transformed by E-B virus in an effort to determine which lymphoid populations are susceptible to virus-induced transformation. In parallel studies, mononucleosis and Burkitt's lymphoma cell lines are being characterized. Malignant and normal lymphocytes from leukemia patients are being established in culture, as are cells from patients with agammaglobu-

linemia and other immunological diseases. Lymphoid infiltrates in tumors are being examined for evidence of cellular immunity.

Major Findings: 1) The newly developed HBLA and HTLA reagents (see previous project report Serial No. NCI-5030) have been shown to be specific markers for B and T lymphocytes respectively, and have been shown to be still present under conditions such as malignancy, immunodeficiency disease, or long-term culture where other markers frequently are lost.

2) Cellular immunity in vitro against some tumors is a T cell phenomenon.

3) Lymphocyte dependent antibody mediated cellular immune reactions are due to B cells.

4) Acute lymphocytic leukemia is a T cell disease, although the sheep red cell rosette assay may be negative.

5) Patients with the Wiskott-Aldrich Syndrome have normal numbers of T cells in the peripheral blood, although the number of sheep red cell rosette forming cells may be very low.

6) The E-B virus appears to preferentially transform B cells.

7) T and B lymphocyte population can be separated by newly developed techniques or modification of existing techniques.

Significance to Biomedical Research and the Program of the Institute: Better means of identifying and separating human lymphocyte subpopulations now permits experiments designed to elucidate immune mechanisms, subclassify clinical disease such as ALL, and provide new information on the pathogenesis of lymphoid diseases.

Proposed Course of Project: Continued studies of malignancies, immunodeficiency diseases, and cellular immune mechanisms in the human.

Honors and Awards: None

Publications:

Edelson, R. L., Smith, R. W., Frank, M. M., and Green, I.: Identification of subpopulation of mononuclear cells in cutaneous infiltrates. J. Invest. Derm., in press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5031 (c)

Title: Human Lymphocyte Markers in Basic Research and in Lymphoid Diseases

Principal Investigator: Richard W. Smith, M.D.

Section:

Lab/Branch: Immunology Branch, DCBD, NCI

Goal: Various clinical and basic studies: comparison of lymphocyte markers in vivo and in vitro in cultured cells, studies of lymphocytes in immuno-deficiency diseases, lymphoid malignancies, cellular immune reactions.

Approach: Five different markers of T and B human lymphocytes are being tested simultaneously in normal lymphocytes and in various pathological states and in in vitro cellular immune responses. Lymphocytes are being exposed to E-B virus in an effort to identify its tropism. Malignant and normal lymphocytes are being established in culture as are cells from patients with agammaglobulinemia in an effort to develop T cell lines and in an effort to have defective B cells from these patients available for experiments designed to induce synthesis of antibody.

Progress: 1) Reliable T and B cell reagents have been prepared. 2) Cellular immunity in vitro has been shown to involve T cells. 3) Lymphocyte dependent antibody mediated cellular reactions involve B cells. 4) ALL is a T cell disease. 5) Wiscott-Aldrich patients' peripheral lymphocytes form SRRC rosettes poorly, but their percentage of peripheral cells is the same as in normals using other measurements of T and B lymphocytes. Thus the SRRC assay, while giving a false low percentage, may reflect the functional deficit in these patients. 6) The E-B virus appears to preferentially transform lymphocyte, or B lymphocytes overgrow T cell cultures. 7) T and B lymphocytes can be separated by selective elimination of one population.

Significance for Cancer Research (NCP Objective 3 Approach 1)

These studies will help elucidate the origin of various lymphoid cancers and show if and how immune mechanisms are involved in their control.

Level of Effort: Professional Man-Years: 1/4

Serial No. NCI-5032(c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Analysis of Cellular and Humoral Immunity Against Histocompatibility and Tumor Specific Antigens

Previous Serial Number: None

Principal Investigator: Richard W. Smith, M.D.

Other Investigators: None

Cooperating Units: Peter T. Mora, M.D., Laboratory of Cell Biology, NCI

Man Years:

Total: 3/8
Professional: 1/8
Others: 1/4

Project Description:

Objectives: To develop improved methods in animal model systems and in humans for the simultaneous analysis of lymphocyte mediated and antibody mediated reactions against transplanted organs or against autochthonous tumor.

Methods Employed: Routine tissue culture methods, preparation of tumor explants and lymphoid cells from various organs. ^{51}Cr labeling on a micro-scale.

Major Findings: 1) Developed a method for ^{51}Cr release cytotoxicity tests in Terasaki microtest plates using only 100-1000 test cells. 2) Developed a procedure for assessing cell mediated cytotoxicity against tumor cells in Terasaki plates by ^{51}Cr isotope labeling. 3) Demonstrated tumor specific transplantation antigen in an SV40 mouse model system.

Significance to Biomedical Research and the Program of the Institute:
A simple means of performing simultaneously ^{51}Cr assays for cellular and humoral immunity against autochthonous tumors has been perfected. This will permit more quantitative and objective evaluation of patients' immune responses to tumor than is possible by present micromethods and will require only minute amounts of tissue and blood.

Proposed Course: The objectives of this project have largely been met and it will be terminated June 30, 1973, in favor of direct application of these tests to clinical and research situations.

Honors and Awards: None

Publications:

Smith, R. W., and Mora, P. T.: Cytolytic microassays and studies of SV40 tumor specific transplantation antigen. Virology 50: 233-246, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5032 (c)

Title: Analysis of Cellular and Humoral Immunity Against Histocompatibility and Tumor Specific Antigens

Principal Investigator: Richard W. Smith, M. D.

Section:

Lab/Branch: Immunology Branch

Goal: To develop improved micromethods in animals and humans for analysis of lymphocyte mediated and antibody mediated reactions against tumors or transplants.

Approach: Tumor cells cultured routinely were labeled in microcultures and ^{51}Cr release or retention was used as a measure of cell viability.

Progress: 1) Developed a method for ^{51}Cr release cytotoxicity tests on 100-1000 cells in the 10 μl wells of microtest tissue culture plates. 2) Adapted a similar procedure for lymphocyte mediated cytotoxicity. 3) Demonstrated tumor specific transplantation antigen in an SV 40 mouse model system.

Significance for Cancer Research (NCP Objective 3 Approach 3)

These studies will permit improved in vitro assessment of the interaction between the immune system and cancer.

Level of Effort: Professional Man-Years: 1/8

Serial No. NIH-5033 (c)
1. Immunology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunotherapy of Human Cancer

Previous Serial Number: None

Principal Investigator: William D. Terry, M.D.

Other Investigators: Lloyd K. Everson, M.D.

Cooperating Units: William Levis, M.D., Dermatology Branch, NCI; Lionel Schour, M.D., Surgery Branch, NCI; Vincent De Vita, M.D., Medicine Branch, NCI; Ayub Ommaya, M.D., Surgical Neurology Branch NINDS

Man Years:

Total:	1/4
Professional:	1/4
Other:	0

Project Description:

Objectives: This project is designed to study various approaches to the immunotherapy of human malignancies.

Methods Employed: Patients will be evaluated for immunologic status and treated with a variety of immunotherapeutic maneuvers. They will be monitored for changes in general immune responsiveness, as well as for specific responsiveness to tumor antigens.

Major Findings: Patients with basal cell carcinomas have been sensitized with DNCB and then treated by application of DNCB in cream to tumor sites. A certain number of tumors regressed completely while others either remained or continued to grow. In general, tumors that are superficial and small are responsive, while those that are nodular, or of larger size, may not respond. In some cases, tumors treated with croton oil (which does not cause an immune response) also disappeared. This raises some questions about the mechanism involved in tumor destruction, and the role of the immune system in this treatment.

Two new protocols have been planned and will be initiated shortly. One involves patients with glioblastoma and will utilize PPD directly into the region of the tumor in patients sensitized to BCG. The other involves patients with stage II and stage III melanoma who will be immunized with an allogeneic cultured melanoma cell line plus BCG.

Significance to Biomedical Research and the Program of the Institute:

Immunotherapeutic approaches provide one new treatment form for certain cancers. These studies will help to define the types of cancers and the approaches that may be most useful.

Proposed Course of Project: Additional patients with basal cell cancers will be treated and the mechanisms involved studied. The two new protocols will be initiated.

Honors and Awards: None

Publications:

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 5033 (c)
Title: Immunotherapy of Human Cancer

Principal Investigator: William D. Terry, M.D.
Section:
Lab/Branch: Immunology Branch
Goal: To study various approaches to the immunotherapy of human malignancy.

Approach: 1) Evaluate DNCB in the treatment of basal cell cancers.
2) Establish protocol for immunotherapy of glioblastoma
3) Establish protocol for immunotherapy of melanoma.

Progress: Patients with basal cell cancers have been sensitized and treated with DNCB. Some, but not all lesions, can be cured with this approach. Protocols for glioblastoma and melanoma have been devised and will be initiated.

Significance for Cancer Research (NCP Objective 6 Approach 1)

These studies will help to define the types of cancers and the types of immunotherapeutic approaches that may be most useful.

Level of Effort: Professional Man-Years: 1/4

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Genetic Control of Antibody Responsiveness to Staphylococcal Nuclease

Previous Serial Number: None

Principal Investigator: Gene M. Shearer, Ph.D. and David H. Sachs, M.D.

Other Investigators: Eugene C. Lozner, M. D.

Cooperating Units: None

Man Years:

Total	1 5/8
Professional:	1 1/8
Other:	1/2

Project Description:

Objectives: Immune responses of inbred mouse strains to a number of immunogens have been shown to be genetically controlled (McDevitt and Benacerraf, Advanc. Immunol. 11: 31, 1969). Two inbred rat strains immunized with Staphylococcal nuclease were found to respond differentially in the production of antibodies which are specific for and inactivate this enzyme (Sachs, unpublished observations). The objective of this project was to investigate in detail the genetic regulation of immune response to nuclease in mice. This species was chosen since the genome of the mouse is better known than that of other rodents. Staphylococcal nuclease is advantageous as a model immunogen, since: (a) its amino acid sequence and three dimensional structure have been characterized; (b) the immunogen is a protein and appears to be more immunogenic than synthetic polypeptides; (c) a very sensitive enzymatic assay has been developed for detecting antibodies to nuclease; and (d) the molecule can be cleaved into a number of fragments which are capable of binding specific subpopulations of antibodies (Sachs, et al., J. Immunol. 109: 1300, 1972). It was also the purpose of this study to extend the studies in the rat to establish whether responsiveness to nuclease is linked to the major Ag-B histocompatibility region. Since genetic control of responsiveness to mitogens has been recently reported in rats (Newlin & Gasser, J. Immunol 110: 622, 1973), possible genetic linkage between nuclease and mitogen response are also being tested.

Methods Employed: A number of inbred mouse strains as well as congenic resistant lines (differing only at H-2) were immunized with nuclease and their sera tested for anti-nuclease activity. Antibody activity was measured by determining the ability of given antiserum to inhibit nuclease hydrolysis of denatured DNA using a quantitative spectrophotometric technique. The number of anti-nuclease hemolytic plaque-forming cells detected in the spleens of immunized mice was determined using a modified Jerne assay. The antibody activity of sera specific for different determinants within the nuclease molecule was determined by hemolysis of tanned sheep erythrocytes coated with fragments of the enzyme. Since no congenic strains of rats are available, linkage studies are being performed by following Ag-B histocompatibility typing and responsiveness to nuclease in successive generations of high and low responder rats. Similar mating studies are being performed in mice to verify linkage to H-2 or other linkage groups. Mitogen-induced stimulation of rat lymphoid cells from different inbred strains are being studied for DNA and protein synthesis.

Major Findings: The major findings made thus far are:

1) Strain-dependent differences were detected in the antibody responses of a number of inbred mouse strains to nuclease. SJL/J mice were the highest responders to nuclease, whereas DBA/1 mice were the lowest responders. (DBA/1 x SJL/J) F_1 mice were also high responders.

2) The response potential appears to be H-2 linked to some extent. Thus, mice which elicited high antibody responses were H-2^{a,s}, intermediate responses H-2^{d,k} and low responses H-2^{b,q}. Confirmation of H-2 linkage was obtained using the congenic lines B10 and B10.A. B10.A (H-2^a) mice were high responders, whereas B10 (H-2^b) mice were low responders. The immune response controlling responsiveness to nuclease in B10 mice was localized to a region in close proximity to the Ir-1 gene, since the 2R and 5R recombinant lines were high and low responders, respectively.

3) Significant differences were detected in the number of IgM plaque-forming cells generated in high and low responder B10 mice, indicating that these H-2 linked genetic differences included differences in the IgM class of antibodies, in contrast to the findings of others using synthetic polypeptides (Grumet, J., Ext. Med 135: 110, 1972).

4) The observations that a wide variation in the response to nuclease among different H-2^k strains of mice and that SJL/J are high responders whereas DBA/1 are low responders (similar to the non-H-2 linked responses of these two strains to (T,G)-Pro--L) raises the possibility that some aspect(s) of the response to this enzyme may not be H-2 linked.

5) Among the various high and low responder strains, all appear to be synthesizing antibodies specific for a determinant contained in the 1-126 fragment of nuclease. Without exception, secondary immunization resulted in the production of antibodies specific for fragment 99-149 as well.

Significance to Biomedical Research and Program of the Institute:

This program is relevant in that it examines the genetic regulation of immune responsiveness to a protein of well defined structure. Although synthetic polypeptides have provided important information concerning response genes, a study of such genes associated with responses to proteins can be more meaningful, since most naturally occurring antigens are complex and since protein antigens are generally better immunogens than are many of the simpler synthetic molecules. It remains to be established whether the immune response gene(s) regulating responses to nuclease (as well as those studied by others) will have any significant bearing on susceptibility to cancer or other diseases.

Proposed Course of Project: Linkage of responsiveness to nuclease to the H-2 complex will be verified in the B10 mice and tested in the SJL/J and DBA/1 example by typing individual F₂ and backcross progeny for H-2 and for responsiveness to the immunogen. Attempts will be made to transfer responsiveness with lymphoid cells in irradiated hosts and to obtain antibody responses in tissue culture systems as a means of examining the cellular basis of low responsiveness. Attention will also be focused on stimulation of various immunological compartments with intact nuclease and nuclease fragments in order to define possible hapten and carrier functions of the original immunogen with respect to immune response gene expression.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 5025 (c)

Title: Genetic Control of Antibody Responsiveness to Staphylococcal Nuclease

Principal Investigator: Gene M. Shearer, Ph.D., David H. Sachs, M.D., Eugene C. Lozner, M.D.

Section:
Lab/Branch: Immunology Branch

Goal: Elucidation of the genetic and cellular aspects of the immune response gene(s) controlling responsiveness to staphylococcal nuclease.

Approach: A number of inbred mouse strains as well as congenic resistant lines (differing only at H-2) were immunized with nuclease and their sera tested for antinuclease activity. Antibody activity was measured by determining the ability of a given antiserum to inhibit nuclease hydrolysis of denatured DNA using a quantitative spectrophotometric technique. The antibody activity of sera specific for different determinants within the nuclease molecule was determined by hemolysis of tanned sheep erythrocytes coated with fragments of the enzyme. Since no congenic strains of rats are available linkage studies are being performed by following Ag-B histocompatibility typing and responsiveness to nuclease in successive generations of high and low responder rats. Similar mating studies are being performed in mice to verify linkage to H-2 or other linkage groups. Mitogen-induced stimulation of rat lymphoid cells from different inbred strains are being studied for DNA and protein synthesis.

Progress: Strain-dependent differences were detected in the antibody responses of a number of inbred mouse strains to nuclease. SJL/J mice were the highest responders to nuclease, whereas DBA/1 mice were the lowest responders. (DBA/1 x SJL/J)F₁ mice were also high responders. The response potential appeared to be H-2 linked to some extent. Thus, mice which elicited high antibody responses were H-2^{a,s}, intermediate responses H-2^{d,k}, and low responses H-2^{b,q}. Confirmation of H-2 linkage was obtained using the congenic lines B10 and B10-A. B10-A (H-2^a) mice were high responders, whereas B10 (H-2^b) mice were low responders. The immune response controlling responsiveness to nuclease in B10 mice was localized to a region in close proximity to the Ir-1 gene, since the 2R and 5R recombinant lines were high and low responders respectively.

Significance for Cancer Research (NCP Objective 3 Approach 4)
This study examines the genetic regulation of immune responses to a protein molecule, which is more complex than most of the immunogens previously examined in this respect. These studies may have some bearing on our understanding of genetic regulation of immunity in general, and possibly to susceptibility to cancer and diseases.

Level of Effort: Professional Man-Years: 1 1/8

LABORATORY OF CELL BIOLOGY

SUMMARY

July 1, 1972 - June 30, 1973

Lloyd W. Law

Some highlights of achievements in the Laboratory of Cell Biology

I. Tumor Associated Antigens in Neoplasms of the Experimental Animal and Man and the Immune Reactions Involved.

An *in vitro* cell-mediated ^{51}Cr release assay across an H-2 barrier using peritoneal exudate cells (PEC) has provided a quantitative and specific means of studying "blocking" factors. Antisera with high titers of cytotoxic antibody regularly, even at dilutions of 1/1000 specifically "block" the lysing effect of sensitized PEC. Of interest is the finding that antisera from completely tolerant or humorally tolerant mice provide no blocking activity. This assay allows us to study "blocking" whether of an antibody nature or as proposed by others of an antigen-antibody complex and to study the cellular site of action of this response. Very preliminary data indicate a "complex" of IgG and H-2 antigen is effective. The ^{51}Cr release assay has been adapted to a syngeneic tumor system (SV40-mKSA fibrosarcoma) in which strong VSTA have been identified.

In our continuing studies of the properties of solubilized, purified Histocompatibility antigens the following pertinent findings are reported: Though preservation of all of the H-2a serologic specificities (public as well as private) have been preserved in digestion and G-150 chromatography, the F2 fraction (known to contain A-2 specificities) induces humoral tolerance but not cell-mediated tolerance to skin and heart grafting. In face of newer evidence that the several functions of T cells are under different genetic influences (in the H-2 complex) we studied GVH reactions, MLR and lymphocyte toxicity produced by our humorally tolerant mice. Cell-mediated reactions of these lymphoid cells, in contrast to normal and completely tolerant mice, were amplified.

We have solubilized and purified VSTA from disassociated cells (ascites and/or tissue culture grown) of 2 neoplasms: RBL-5, a leukemia, and mKSA, an SV40-induced fibrosarcoma; both have strong VSTA. Activity of the RBL-5 antigen is specific and is detected in Cr51 *in vitro* inhibition and provides a specific immune state in syngeneic mice against neoplasms sharing FMR antigens. The F2, G150 Sephadex fraction, is the effective fraction. Sensitive assays for the detection of infectious MuLV in our antigen preparations are consistently negative, providing evidence that RBL-5 antigen is a new cellular antigen (virus specified) and not a viral envelope antigen. The SV40 specified VSTA has also been solubilized and purified, again yielding an F2

fraction with specific immunogenic properties. CM (crude membrane) CS (crude soluble) and an F2 fraction immunized against the grafting of the syngeneic mKSA neoplasm. Assays using the complement-mediated cytotoxic inhibition assay (^{51}Cr release) as expression of TA however indicates that the antigens described above in in vivo studies are not identical with those eliciting cytotoxic antibody production. This dichotomy of response demands caution in interpreting the meaning of in vitro expressions of antigens.

The yield of soluble H-2^a antigens (alloantigenic activity) and tumor specific antigens (TA) has been in the range of 60-80% and 4-6% respectively (protein); nonetheless chromatography on Sephadex G150 showed that all of the activity in cell preparations was concentrated in a distinct peak of molecular wt - 5000 and no lipid was found in the preparations. This is quite different from results of others who have reportedly solubilized tumor antigens (Rapp, Smith and Mora, Herberman, Baldwin). We are therefore using different approaches to solubilize and obtain greater yields of TA. Some better success has been achieved using citraconic anhydride. This method should yield dissociated entire molecules from the membrane. The solubilized H-2^a and TA following citraconylation were doubled in yield but were inactive in ^{51}Cr inhibition. A concerted search for other solubilizing reagents more readily dissociable at neutral pH is now in progress.

Membrane antigens have been detected by classical rejection procedures on 3 fibrosarcomas induced in vitro by the antigenically related slow viruses, Visna and PPV. Viral genomes were recovered from these neoplasms by cultivating with sheep testis cells and extensive assays for other DNA - and RNA-containing were negative. In contrast to the VSTA of other RNA viruses, these did not cross-react. They resembled in this respect the TSTA of chemical induced neoplasms.

In the mouse MSV pseudotype viruses induced tumors that are not malignant and that continuously produce infectious MSV; these tumors do contain mesenchymal elements malignant in character and we have been able to select a malignant hemangiosarcoma, XM-1, that is non-productive of infectious viruses but contains defective MSV rescuable by helper RLV. In an attempt to further characterize the MSV-specified VSTA on this malignant line we have established a series of in vitro clones each of which has been studied for its virus productivity, oncogenic potential and its antigenic properties in in vivo rejection experiments. Heterogeneity of all these characters was observed in these clones and the following results were obtained:

a) There was no relationship between virus production (MSV and MuLV) and immunogenicity or sensitivity to the rejection response of specifically immunized syngeneic mice; yet all antigens cross-reacted.

b) One clone, though being oncogenic and releasing infectious virus was found not to contain VSTA; another clone was capable of immunizing against other clones and the parental line but itself was not tumor producing.

c) These results signify that virus-specified new cellular antigens are found on RNA virus-induced neoplasms that are analogous to those of DNA virus induced neoplasms. This does not rule out the likelihood that viral structural antigens are also effective inhibitors of growth.

An inhibition assay has been developed for studying the specificity of cellular cytotoxic responses in vitro against Gross virus-induced leukemias in rats and MSV in mice. The specificities of these antigens are quite different from the specificities of the antibodies and the detected antigens are species specific and may be related to antigens induced by endogenous C-type viruses of each species.

Among Gross virus induced leukemias of rats and mice antibodies were prepared and identified that could detect at least 4 different surface antigens: GCSAa, GCSAb, GSCAc and G₁X. The significance of these serologic reactions in terms of in vivo induction or inhibition of tumors needs to be defined.

Serologic analyses of antigenic specificities on SV40-induced tumor cells have been studied using the Isotopic Antiglobulin Technique (IAT). Two antigenic specificities were identifiable and attempts were made to correlate these with in vivo assays for VSTA; only one, previously identified as TSSA seemed to correlate. In still another tumor system FBL-3, a Friend virus induced leukemia, IAT detected 3 specificities (using syngeneic immunization with FBL-3). One specificity was unique for FBL-3, another was common for the FMR groups and a third appeared to be fetal.

It has been observed that primary plasmacytomas are easily transplantable into "primed" (intraperitoneal injections of mineral oil) syngeneic mice at cell concentrations of 10^2 to 10^3 cells; all 15 tumors not transplantable to normal mice grew progressively in "primed" recipients. Abrogation of immunity was also seen in "primed" mice against a plasmacytoma known to contain strong TSTA. The mechanism of "priming" is being studied.

Studies of Alpha-fetoprotein (α FP).

A sensitive double-antibody radioimmunoassay has been used to detect normal levels of serum α FP and thus may allow useful monitoring for selected neoplastic diseases. In a survey of α FP elevation in other diseases it was found that 10/44 with pancreatic cancer, 15/80 gastric cancer, 9/197 colon cancer, 16/50 lung cancer, 34/48 embryonal cell cancer, 2/8 bile tract cancers but 0/14 esophageal and 0/50 breast cancers were positive above the normal range. Also those patients with lung carcinoma negative by double diffusion testing were positive in the new assay.

Tumor immunity studies in man have revealed the following interesting observations:

a) Whereas skin reactivity to recall antigens in acute leukemia patients has been found to be intact even in relapse, delayed skin reactivity to crude membrane extracts to both autologous and allogeneic

"blast" cells and of Burkitt lymphoma cells has correlated with remission status.

b) Skin reactivity of cancer patients has been studied using antigens prepared from various neoplasms with the following findings: 1) antigens of leukemic cells (acute myelogenous) solubilized by frequency sonication and chromatography (G-200 Sephadex) were found reactive even upon further purification on DEAE cellulose and gel electrophoresis. 2) The skin reactive antigen from intestinal cancer has been found to be a contaminant of CEA preparations of several sources but distinct from the CEA of Gold. 3) Soluble skin reactive antigens from carcinomas of the breast and lung and from melanoma are not distinguishable from skin reactive antigens obtained from homologous normal tissues.

c) Extensive *in vitro* assays are being performed to measure the tumor associated antigens of human neoplasms, the leukocytes of cancer patients in a leukocyte migration assay have been inhibited by hypertonic salt extracts of autologous or allogeneic tumors of the same histologic type. Antigens like PPD and SKSD have been shown to cause inhibition of migration of the leukocytes of skin test positive individuals.

II. Studies in Immunochemistry

A series of mouse myeloma proteins with antigen binding activity have been developed in this laboratory. We are currently studying two groups of these proteins in an effort to 1) determine the structure of the antibody combining site at the molecular level and 2) determine the degree of variability possible among immunoglobulins binding the same haptenic determinant.

In continuing studies of the crystallization of the pepsin Fab from the phosphorylcholine binding myeloma protein McPC 603, we have demonstrated that both the Fab in solution and the crystal bind hapten stoichiometrically. X-ray crystallographic studies have recently produced a 4.5 Å resolution map of the Fab in which several features of the immunoglobulin molecule are readily apparent. Two large globular domains representing the variable and constant portions of the molecule are seen. Each domain is divided into two halves which are presumably the light and heavy polypeptide chains. Using a heavy atom derivative of phosphorylcholine the antigen binding site has been located. The hapten is located in a large cleft between the light and heavy polypeptide chains at the tip of the variable domain. We are currently determining the primary structure of the polypeptide chains. Using a Beckman Sequencer we have been able to determine the sequence for approximately 1/2 of the light chain and 1/3 of the heavy chain. The primary sequence determination plus the high resolution may will enable the construction of a molecular model. We have also recently crystallized the Fab from a galactan binding myeloma protein which we plan to use for a comparative study. To approach the degree of variability among antibodies binding the same hapten we are currently studying two groups of proteins 1) those binding β -(1-6)-galactan and 2) those binding phosphorylcholine. We have recently identified and purified by affinity chromatography 6 galactan binding proteins. Each of these proteins possesses an individual antigenic

specificity (idiotype) indicating primary structural differences. Preliminary sequence studies have shown that the light chains from all six proteins are identical for the first 23 residues. The heavy chains from 4 of the proteins are identical for 30 residues and the other 2 have single substitutions. In contrast a study of 4 phosphorylcholine binding proteins has revealed a different pattern of variation. Two of the proteins, H8 and T15 are idiotypically identical and have the same primary structure through the first two hypervariable regions of the light chain. Two others, M 603 and M 167, which are idiorotypically different have quite different light chain sequences from each other and from H8 and T15. The heavy chains from these proteins however, are identical through the first hypervariable region with the exception of a single substitution in the M167 chain.

We have determined the complete amino acid (aa) sequence of the homogeneous light chain from an anti-p-azobenzoate antibody of rabbit immunoglobulin by the sequencing of tryptic and peptic peptides. The heavy chains from this same antibody show heterogeneity, however. Thus different heavy chains may be paired to a single light chain to generate related antibody.

Studies were made of the relationship of the antibody combining site to the primary structure of the rabbit immunoglobulin molecule. Peptides containing tyrosyl residues from the site of anti-p-azobenzoate antibodies were isolated from the light chain of the molecule and tyrosine that contributed to the site was placed at position 96 (by complete aa sequence). This corresponds with the 3rd hypervariable region of the light chain.

A genetically marked recombinant type of γ -heavy chain from the rabbit (detected serologically) has been used to provide direct proof that the VH (variable unit) and CH (constant unit) are separate genetic units. Recombinant- $\alpha 2$ now linked with A14; normal - $\alpha 1$ linked with A 14; α is located in variable region and A is located in constant region of γ -chain. Extensive chemical analyses of the area of the primary sequence carrying determinants at this point signifies that this event appears to be recombination and not point mutation.

III Macromolecular and ultrastructural biology; oncogenic virus studies.

Patients with xeroderma pigmentosum (XP) are unusually sensitive to sunlight and susceptible to skin neoplasms. XP cells are UV-sensitive in vitro; these appeared as likely candidate cells in which to look for C-type RNA virus and to study susceptibility to transformation with FSV and Ki MSV, sarcomagenic viruses. In extensive studies C-type viruses could be activated but these cells in vitro were more sensitive to both sarcomagenic viruses.

Fibroblasts derived from patients with Lesch-Nyhan syndrome were fused with a non-producer rat cell carrying an MSV genome. These test cells are being used to search for C-type RNA viruses from cells of

human Hodgkins disease and other neoplasms. Putative C-type viruses should act as helper for rescue of the MSV genome from the hybrid cells.

A new leukemogenic virus (S-MuLv) has been isolated from reticular cell neoplasms of SJL strain mice; this virus is antigenically unrelated to any of the known leukemogenic viruses. It has a unique envelope antigen and induces distinct cellular surface antigens.

Intracisternal (IC) - and intracytoplasmic (ICy) A particles found in a variety of normal and tumorous tissues of several species have no known oncogenic activity; the latter is thought however to be a precursor of the mammary tumor virus. It has now been established that IC particles have an associated 60-70 S RNA and they have an enzyme activity having the characteristics of a reverse transcriptase - thus giving evidence for repression of a viral genome. The several RNA subunits obtained are being studied for their functional significance. A detailed study of the structural proteins of ICy particles show 3 major bands (gel electrophoresis) that differ from the 5 major bands of the mammary tumor virus.

A tissue culture line of a lymphoma induced by Herpesvirus saimiri (HVS) has been shown to possess an HVS antigen and enzyme activity analogous to the reverse transcriptase of oncornaviruses; no virus-like particles have been identified as yet in these preparations.

Abelson leukemogenic virus (A-MuLv) a C type RNA virus complex containing MLV and LDH has been shown to induce rapidly (1-2 months) 15-20% plasma cell neoplasms in BALB/c mice. Virus infection must follow intraperitoneal treatment with mineral oil. These plasma cell neoplasms resemble those previously described (without virus infection) in producing different myeloma proteins.

Glycosyltransferase content and activity were studied in tissue culture cells transformed by an RNA virus (MSV) in order to compare changes associated and reported previously with DNA virus transformations. The specific alteration in ganglioside biosynthesis - that is reduced N-acetylgalactosaminyl-transferase activity - occurred subsequent to transformation. The relationship of these changes to in vitro and in vivo growth patterns is under study. Decreased enzyme activity was also seen in 2 other cell lines and persists in single cell clonal progenies.

IV. Somatic Cell Genetics

In the course of studies to obtain replicating cell strains from non-dividing cell types (such as neurons and heart cells) it was found that the majority of hybrid strains were "reverse segregants" - that is in crosses of mouse x man - these hybrid cells lost mouse rather than human chromosomes. Those cell lines have valuable applications to study modified cell surface antigens, host range specificity of oncogenic viruses and genetic linkage group analysis of the mouse.

Further analyses of the character of mitochondrial DNAs (mt-DNAs) in human-rat, human-mouse hybrid cell strains have revealed that human and mouse mt-DNA are quite capable of forming hybrid mt-DNA molecules; in some cell strains, specifically mouse brain cells x a transformed human cell line. The mt-DNA is exclusively of hybrid or recombined DNA. The existence of recombinant mt-DNA (in apparent absence of selection) provides a means of genetic analyses of mt-DNA. Transcriptional processes as well as function may now be studied in other systems using mt-ribosomal RNA and mt-tRNA assays.

Patients with the autosomal recessive disease xeroderma pigmentosum (XP) usually die of metastatic skin cancer, skin fibroblast from XP patients are known to be deficient in UV-induced ³H-thymidine (TdR) incorporation (DNA repair synthesis). An endonuclease defect has been inferred as the cause of this defect. A series of 13 XP patients were studied, making fusions of fibroblasts to produce heterokaryons among them and analyzed TdR incorporation. These patients fell into 4 distinct complementation groups - that is crosses restored the repair syntheses. Each complementation group had its own DNA repair rates - not specifically related to clinical manifestations of the disease. It is hoped that those assays will lend themselves to the isolation of cytoplasmic extracts and ultimately to purification of the factors involved in DNA repair.

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PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: I. Chemical structure of immunoglobulin molecules
II. Purification and biochemical characterization of
soluble murine H-2 antigens and tumor specific antigens

Previous Serial Number: Same

Principal Investigator: Dr. Ettore Appella

Other Investigators: Dr. R. Mage (NIAID)
Dr. John Inman (NIAID)
Dr. Oliver Roholt, Roswell Park Memorial Inst.
Dr. David Pressman, Roswell Park Memorial Inst.

Man Years:

Total: $2\frac{1}{2}$

Professional: 1

Other: $1\frac{1}{2}$

Project Description:

1. Mouse immunoglobulins

Intensive studies have been devoted to obtaining information on the chemical structure of IgA. The alpha chain of MOPC 511 has been studied and was shown to be of about 450 amino acid residues with an available amino-terminus, glutamic acid and tyrosine at the carboxy-terminus. Cyanogen bromide cleavage resulted in the separation of seven fragments, which were purified by a combination of gel filtration and ion exchange chromatography. Their molecular weights were determined by chromatography on a calibrated agarose column and by SDS gel electrophoresis. On the basis of the methionine content and total amino acid composition of the intact chain, the cyanogen bromide fragments account for the entire chain.

One characteristic of the alpha chain is a large number of cysteine, fifteen residues per mole, compared to a $\sqrt{G}2a$ mouse immunoglobulin which has twelve residues per mole. The extra cysteine must be representative of the IgA class. There is also the important question of the positions of the cysteine and placement of the disulfide bridges which was raised upon finding ten cysteine in a fragment of about 210 amino acids. The complete primary sequence of this protein is under investigation, and we hope it will shed light on this interesting problem.

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Another alpha chain, MOPC 47A, has been studied. Previous work showed that its molecular weight is about 40,000 suggesting deletions of about 100 amino acids. Cyanogen bromide cleavage and separation of the resulting fragments have accounted for the entire chain. This verified the absence of 100 amino acids which are found in 511. Since 47A heavy chain has glutamine as the carboxy-terminus whereas 511 has tyrosine and since the carboxy-terminal octapeptide from cyanogen bromide cleavage of 511 is not present in 47A, we conclude that at least part of the deletion in 47A is in the carboxy-terminus portion of the chain. The significance of this deletion is still unknown, but it could have resulted from an unequal crossover between pairs of alleles determining mouse IgA heavy chain or an abnormality of the transcription process.

Another aspect of the study of the primary sequence of IgA is the possibility of defining subgroup variability of the mouse heavy chain. So far only one V_H sequence has been reported for mouse immunoglobulin, MOPC 173, and it shows considerable homology with $V_{H III}$ sequence of human heavy chains. Determination of the first 52 amino acids of 47A seems to indicate that this V-region belongs to the same V_H subgroup as MOPC 173. Although not enough sequence data are available to make a definite statement, this might indicate that the major subgroup in rodents is of the $V_{H III}$ type.

MOPC 511 has been found to have antibody activity for choline and phosphorylcholine. Another tumor, MOPC 167, has similar antibody activity but differs by an idiotypic determinant. Comparison of the two proteins at the level of fingerprinting of the tryptic peptides shows a large degree of similarity; however, amino acid sequence of the amino-terminal ends of both light and heavy chains showed substitution of lysine (511) for aspartic acid (167) in the light chain at position 11 and valine (511) for leucine (167) in the heavy chain at position 4. Detailed sequence analyses of both proteins should give insight to where other differences occur, the hypervariable region being of particular interest.

2. Rabbit immunoglobulins

Continuing efforts have been devoted to obtaining the complete amino acid sequence of the light chain from an anti-p-azobenzoate antibody of restricted heterogeneity. This goal has been achieved by the isolation and sequencing of tryptic and peptic peptides. It appears that the light chain from this antibody is indeed homogeneous. The heavy chain from the same antibody was also studied by cyanogen bromide cleavage. Six fragments were identified from the total reduction and alkylation of C1, a fragment which consists of the amino-terminal half of the heavy chain. From the known positions of the half-cystine residues of C1 it appears that heavy chains having different distributions of methionine are present in the antibody. In other words the heavy chain is not homogeneous. Since we have shown that the light chain is homogeneous, this indicates that a limited

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set of different heavy chains may be paired to a single light chain to generate related antibody molecules.

Another aspect of our work is studying the relationship of the antibody combining site to the primary structure of the molecule. Peptides containing tyrosyl residues from the site of anti-p-azobenzoate antibodies were isolated by means of the paired-iodination procedure. Only ^{the}light chain of this antibody was labeled, and the tyrosine that contributes to the site was placed at position 96 by complete amino acid sequence. This is interesting because it corresponds with the third hypervariable region of light chains.

A recombinant type of γ -heavy chain has been reported (Mage et al. (1971) Nature, 230, 63). The normal progeny was represented by a1 linked to A14. In the recombinant type a2 was now linked to A14. The A14 and A15 allotypes correlate with a threonine-alanine interchange at position 309 of the constant region of rabbit γ -chain (Appella et al. (1971) Proc. Natl. Acad. Sci. (USA) 68, 1341). The recombinant type, which serologically was A14, had threonine in position 309 as expected. Amino acid compositions were carried on Fd fragments derived from the normal and recombinants type molecules. The data obtained clearly support the assignment of a2 allotype to the recombinant type. These amino acid compositions do not define, however, the peptides carrying the antigenic determinant of the a allotypes. To gain information about this important aspect of the problem we tried to identify these peptides either from Fd or the heavy chains of restricted antibodies. Tryptic peptides from a1, a2, and a3 partially cytraconilated heavy chains were purified from a Sephadex G-75 and immunoabsorbent and were shown to inhibit specifically the precipitation of anti-a locus antibodies with IgG in Ouchterlony gel diffusion. The amino acid sequence studies of these peptides are now in progress. This should definitively assign the residue position of the determinants and define more exactly the correctness of the assignment of a2 to the recombinant.

3. H-2 alloantigens and tumor specific transplantation antigens

Enzymatic digestion with papain has been used to solubilize murine histocompatibility antigens (H-2^a) and tumor specific antigen of an SV40 virus-induced fibrosarcoma and Rauscher induced mouse leukemia (RBL-5). The yield of soluble alloantigenic activity was about 60-80% for H-2^a, however, for both tumor lines only 2-4% of the activity could be solubilized. Chromatography of both preparations on Sephadex G150 showed that all the activity was concentrated in a distinct peak of molecular weight approximately 50,000. No lipid was found to be present in this fraction. The use of immunoabsorbent to further purify this fraction is now in progress.

Using a different approach, citraconic anhydride has been employed to achieve solubilization of H-2^a and tumor specific antigens. In contrast to enzymatic digest, solubilization by this method is achieved by alteration

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of the overall charge, mostly at the epsilon amino group of lysine residues, and, thus, should dissociate entire molecules from the cell membrane. After citraconylation about 40% of the membrane proteins were in soluble form for H-2^a and only about 4-8% for the tumor lines, both antigenically inactive in the ⁵¹Cr inhibition test. Deblocking of the ε-amino group has been tried by exposure at pH 6.4, but only about 10% of the alloantigenic activity for H-2^a could be restored and none for the tumor specific antigens. A search for a different solubilizing reagent more readily dissociable at neutral pH is now in progress.

Publications:

- Appella, E., Inman, J. K.: The primary structure of rabbit and mouse immunoglobulin light chains: The structural basis for allotypy. In Contemporary Topics in Molecular Immunology (Inman, F. P., ed.), Plenum Press, Vol. II, 1973.
- Potter, M., Mushinski, E. B., Rudikoff, S., Appella, E.: Antigen binding myeloma proteins in mice that bind the same hapten. Presented at the Third International Convocation on Immunology, Buffalo, New York, 1972.
- Law, L., Appella, E., Strober, S., Wright, P., and Fischetti, T.: Induction of immunological tolerance to soluble mouse histocompatibility-2 antigens. Proc. Nat. Acad. Sci. (USA) **69**: 1858-1862, 1972.
- Law, L., Appella, E.: Immunogenic properties of solubilized tumor antigen from an RNA virus-transformed neoplasm. Nature, New Biology, 1973. (in press).
- Robinson, E. A., Appella, E., and McIntire, R.: Structural studies of a heavy chain from a mouse immunoglobulin M with antibody activity. J. Biol. Chem., 1973 (in press).
- Carta-Sorcini, M., Appella, E., Inman, J. K., and Mage, R. G.: The immunoglobulins derived from a V_H-C_H recombinant rabbit and its normal relatives. Immunochemistry, 1973. (in press).

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3229

Title: 1. Chemical Structure of Immunoglobulins

2. Biochemical characterizations of soluble murine H-2 alloantigens and tumor specific antigens

Principal Investigator: Ettore Appella

Section: Office of the Chief

Lab/Branch: Laboratory of Cell Biology, DCBD

Goal: By studying the molecular structure of immunoglobulins and cell surface antigens we hope to gain further insight into the mechanism of immunological processes.

Approach: The primary structure of mouse myeloma proteins and rabbit antibodies of restricted heterogeneity has been studied. Conventional techniques of protein sequencing include amino acid analyses, enzymatic digests and chemical splittings, and peptide isolation, purification, and sequencing. Sequencing is carried out both by manual methods and with an automated machine using the basic Edman degradation reaction which selectively removes the amino-terminal amino acid. New approaches to this important reaction such as modifications of the chemical techniques used and of the subsequent identification procedures of the amino acids are being investigated. Enzymatic digests with papain of membranes from H-2^a mouse spleen cells and virus-transformed cells solubilize the cell surface antigens. These antigens are chromatographed and elute in the same area as single, discrete peaks of molecular weight 50,000. Preparations of specific immunoadsorbents to further purify the antigens is our present approach to enable us to study their primary structure.

PROGRESS: A complete primary sequence of a rabbit immunoglobulin light chain and a nearly complete sequence of a mouse α -heavy chain has been obtained. This should give some insight into the problems of distinctive features of the primary structures of different classes in the same species, e.g. IgA, versus IgG, or the same classes in different species, e.g. μ chains in rabbit versus human and mouse. The sequenced rabbit light chain derived from an anti-p-azobenzoate antibody had a tyrosine at position 96 labeled to indicate a combining site. This position corresponds to the third hypervariable region found in light chains of other species. Solubilization of H-2 and tumor specific antigens has been achieved. We were thus able to separate the antigens from other components of the cell surface which do not contribute to antigenic activity. Therefore, when methods for further purification have been developed, we will be able to study the primary structure.

Significance for Cancer Research (NCP Objective 3 Approach 4,5)

Level of Effort: Professional Man-Years: One

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on oncogenic RNA viruses.

Previous Serial Number: Same

Principal Investigator: Dr. Kenneth S. S. Chang

Other Investigator: Dr. L. W. Law (C:LCB-3200)

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

1. Interaction of genetically deficient human cells with oncogenic RNA viruses.

Skin fibroblasts cultured from patients afflicted with genetic diseases manifest unique characteristics which can be exploited in tumor virus studies:

A) Xeroderma pigmentosum (XP): Since the disease is associated with unusually high sensitivity to sunlight and a high incidence of skin carcinogenesis, it would be of interest to examine: a) if any C-type RNA virus could be activated from these cells which are UV-sensitive in vitro; b) susceptibility to transformation of these cells by known tumorigenic viruses such as Kirsten murine sarcoma virus (KiMSV) and feline sarcoma virus (FSV) as compared with cells obtained from heterozygotes and apparently normal individuals; and c) effect of 4-nitroquinoline-1-oxide (4NQO) (which is a carcinogen and an inducer of unscheduled DNA synthesis as is UV light) on the transforming activity of KiMSV and FSV.

It was found that: a) so far no C-type RNA virus could be activated from these cells; b) compared with normal age-matched controls, the XP cells particularly those derived from patients with neurological complication were relatively more susceptible to FSV transformation, and cells from some XP patients and

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heterozygotes exhibit high FSV/KiMSV ffu ratios; and c) pretreatment of cells with 4NQO at a dilution neither toxic nor directly transforming rendered the XP cells more susceptible to transformation by KiMSV and FSV whereas no such enhancing effect of 4NQO at the same concentration was observed with cells derived from a normal subject.

B) Lesch-Nyhan syndrome (LNS): Fibroblasts derived from these patients were used for Sendai-virus mediated fusion with an MSV non-producer rat cell (KiMSV-NRK) which was originally transformed by KiMSV. An advantage was taken of the facts that LNS cells which were deficient in hypoxanthine-guanine phosphoribosyl transferase activity could be eliminated from a selective (HAT) medium, and that the growth of non-producer KiMSV-NRK cells could be restricted in azaguanine-containing medium. A clone of hybrid cells so obtained was studied by chromosome analysis. Susceptibility of these cells to human poliovirus and other animal viruses by plaque assay was examined. The presence of the MSV genome in the hybrid was confirmed by rescue assay using various leukemia viruses as helpers. Further studies are in progress: a) to examine the influence, if any, of the human cell component on the expression of the KiMSV genome in the hybrid cells; and b) to use these hybrid cells carrying MSV genome and human cell receptors in a search for C-type RNA viruses from human tissues such as Hodgkin's disease lymph nodes and spleens by the ability of the putative human C-type viruses to act as a helper for rescue of MSV genome from the hybrid cells.

2. Further studies on the C-type RNA virus (S-MuLV) isolated from the SJL/J strain of mouse.

As a model for study of the possible role played by oncogenic RNA virus in the pathogenesis of human Hodgkin's disease, SJL/J strain of mouse which manifests a high frequency of spontaneous Hodgkin's-like reticulum cell neoplasm (Type B) has been the subject of study.

A) Virological and immunological studies: The C-type RNA virus (S-MuLV) isolated from a diseased mouse was shown to have N-tropism, reverse transcriptase, and murine gs antigens. The envelope antigen was further characterized by neutralization tests and immunoelectronmicroscopic studies (in collaboration with Dr. Aoki, NCI). Antiserum against the virus was prepared by 7-8 weekly immunizations of adult SJL/J mice with X-irradiated suspension of syngeneic lymphocytic leukemia (D-1) which was induced by the virus. This serum did not cross-react with viral envelope antigens of Gross, Moloney or Rauscher, or those produced by

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BALB/c plasmacytoma and BALB/3T3 S2 (spontaneously transformed) cells. It reacted only with a population of AKR spontaneous leukemia virus, and a radiation-induced leukemia virus of an A strain mouse as well as with the homologous virus (S-MuLV). The cell surface antigen on D-1 was also shown to be unique by immunoelectronmicroscopic studies. It did not cross-react with either antiserum against PC1 antigen of plasmacytoma or W/Fu rat anti-(C58NT)D serum which detects G_{IX} alloantigen as well as G and FMR cell surface antigens. The D-1 cells did not absorb appreciably the cytotoxic antibody from the W/Fu rat anti-(C58NT)D serum under conditions where C57BL/6 E₈ G2 leukemia cells (Gross-virus induced) could do so. Thus it would appear that S-MuLV has a unique envelope antigen and induces distinct cellular surface antigens not shared by those induced by many other oncogenic RNA viruses. Immunologic relationship of these antigens with those present on the spontaneously occurring Hodgkin's-like reticular neoplasm will be investigated.

B) Pathogenicity studies: Injection of the isolated virus, S-MuLV, into neonatal SJL/J mice induced a lymphocytic leukemia, the latent period of which was shortened from 5 months (obtained in previous experiments) to 2 1/2 months when a concentrated virus suspension was used. A type A reticulum cell sarcoma was found in the subcutaneous tissue of an SJL/J mouse that was transplanted with a tumor mass arising at the site of inoculation of MSV (S-MuLV) in an antilymphocyte serum-treated mouse. However, so far no type B lesion has been induced by the isolated virus. Among newborn mice of various strains tested (BALB/c, C57BL/6 and AL/N), a mouse of AL/N strain developed a lymphocytic leukemia 8 1/2 months after injection of unconcentrated virus. Since it was suspected that development of lymphocytic leukemia may mask the slowly developing type B lesions which do not depend on the thymus cells for their development, neonatal thymectomy was performed on SJL/J and BALB/c mice and a suspension of concentrated S-MuLV was injected. These mice are still under observation for development of any significant neoplasms.

Publications:

S. S. Chang: Effect of radiation on murine sarcoma virus genome rescue activity. J. Gen. Virol. (accepted for publication).

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 5550

Title: Interaction of Genetically Deficient Human Cells With RNA Tumor Viruses

Principal Investigator: Kenneth S. S. Chang, M.D., Sc.D.

Section: Viral Oncogenesis

Lab/Branch: Cell Biology, DCBD

Goal: To find any link between the high incidence of carcinogenesis in xeroderma pigmentosum patients and the susceptibility of their cultured fibroblasts to C-type RNA viral oncogenesis.

Approach Since the disease, xeroderma pigmentosum (XP), is associated with unusually high sensitivity to sunlight and a high incidence of skin carcinogenesis, it would be of interest to examine: 2) if any C-type RNA virus could be activated from these cells which are UV-sensitive in vitro; b) susceptibility to transformation of these cells by known tumorigenic viruses such as Kirsten murine sarcoma virus (KiMSV) and feline sarcoma virus (FSV) as compared with cells obtained from heterozygotes and apparently normal individuals; and c) effect of 4-nitroquinoline-1-oxide (4NQO) (which is a carcinogen and an inducer of unscheduled DNA synthesis as is UV light) on the transforming activity of KiMSV and FSV.

Progress: It was found that: a) so far no C-type RNA virus could be activated from these cells; b) compared with normal age-matched controls, the XP cells particularly those derived from patients with neurological complications were relatively more susceptible to FSV transformation, and cells from some XP patients and heterozygotes exhibit high FSV/KiMSV ffu ratios; and c) pretreatment of cells with 4NQO at a dilution neither toxic nor directly transforming rendered the XP cells more susceptible to transformation by KiMSV and FSV whereas no such enhancing effect of 4NQO at the same concentration was observed with cells derived from a normal subject.

Significance for Cancer Research (NCP Objective 3 Approach 2)

This study may throw light on the relationship between the nature of genetic defect responsible for XP (i.e., defect in endonuclease and possibly errors in DNA replication processes) and mechanism of neoplastic transformation by C-type RNA viruses.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-5561

1. Laboratory of Cell Biology
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Development of Various In Vitro Methods to Detect Cell Mediated Immunity

Previous Serial Number: None

Principal Investigator: Dr. James M. Cohen

Other Investigators: Dr. Lloyd W. Law (NCI-3200)
Dr. S. S. Yang (NCI-4833)
Dr. S. B. Howell (NCI-5560)

Man Years: Total - 2
Professional - 1
Other - 1

Project Description:

Using a ^{51} chromium release cell mediated cytotoxicity and a micro-cytotoxicity assay (modified from Hellström's colony inhibition test), we studied the following problems:

1. The effect of hyperimmune enhancing alloantiserum on cell mediated immunity. In this study, we showed that alloantiserum was able to block cell mediated immunity in a specific, quantitative and highly reproducible fashion. Presently, we are attempting to isolate the blocking factor and also define the cell at which blocking takes place.

2. The effect of tolerant mouse serum on cell mediated immunity. Serum from cell mediated and humorally tolerant mice had no effect on cell mediated immunity in vitro using the ^{51} chromium release assay. Further work will attempt to determine whether tolerant animals possess cytotoxic lymphocytes.

3. The changing antigenicity of a Polyoma Induced Fibrosarcoma. A fortuitous disappearance of tumorigenicity in a tissue culture cell line has been investigated with respect to retention of tumor specific antigens using the microcytotoxicity assay as well as in vivo studies.

4. The correlation of the ^{51} chromium release cell mediated cytotoxicity assay and microcytotoxicity was studied. The developmental kinetics of syngeneic tumor immunity were studied using both assays and comparing their results to the animals' ability to resist tumors at various times after immunization.

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 5561

Title: Development of various in vitro methods to detect cell mediated immunity

Principal Investigator: James M. Cohen

Section: Office of the Chief

Lab/Branch: Laboratory of Cell Biology, DCBD

Goal: Using in vitro assays, we are attempting to study blocking of cell mediated immunity (CMI) and its relationship to immunological enhancement

Approach Using a ⁵¹chromium release cytotoxicity assay, we studied the effects of hyperimmune alloantiserum on CMI and its ability to enhance tumors across H-2 barriers. In addition, the same assay system was used to study the effect of tolerant animals' serum on CMI. Using a microcytotoxicity assay, the developmental kinetics of syngeneic tumor immunity are being studied and this assay and the ⁵¹chromium release assay are being compared.

Progress: Hyperimmune enhancing alloantiserum specifically, quantitatively and reproducibly blocked CMI in the ⁵¹chromium release assay. Serum from cell mediated and humorally tolerant animals did not block CMI whereas serum from hyperimmune animals did block.

Significance for Cancer Research (NCP Objective 6:4 Approach 1.0)

Level of Effort: Professional Man-Years: 1

Serial No. NCI-5552

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PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Production and analysis of hybrid somatic cells: their application to some problems of importance in biology and medicine

Previous Serial Number: Same

Principal Investigator: Hayden G. Coon, Ph.D.

Other Investigators: Dr. Igor B. Dawid, Carnegie Inst., Baltimore
Dr. Ivan B. Horak, Carnegie Inst., Baltimore
Mr. G. David Trisler (C:LCB)
Dr. Peter O. Kohler (NICHD:RPL-1)
Dr. Jay Robbins and Dr. Kenneth Kraemer (NCI:D)

Cooperating Units: None

Man Years: Total - 2.00
Professional - 1.00
Other - 1.00

Project Description:

A) Introduction.

Our unit has been doing research aimed at learning how to manipulate the various forms of genetic material in vertebrate cells and the development of systems in which ordinary genetic manipulations such as mutation, recombination, etc. can be practiced with the somatic cells of vertebrate tissues. It is obvious that a more complete ability to manipulate the genetics of vertebrate cells must precede and will probably prove to be the means by which we shall come to understand the biochemical mechanisms at work in normal and malignant cells.

A new research area has emerged in the past ten years which is now called "Somatic Cell Genetics" or simply, "Cellular Genetics." The production, husbandry, and analysis of hybrid cells are vital components of this new field. The chief areas required are: 1) sophisticated animal cell culture; 2) modern methodology for analysis of chromosome morphology and chemistry; 3) biochemical analysis - our work has consisted of assays of enzyme activities purification and identification of certain protein products, e.g., immunoglobulin and collagen, and the detection of characteristic nucleotide sequences by DNA-DNA and RNA-DNA molecular hybridization. Immunochemical techniques have become increasingly important in our work especially in studying antigens specific to the nervous system expressed in hybrids of fibroblasts and nerve

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cells. It is the combination of these various activities in a single laboratory working on a common group of problems centering upon the unique genetic material found in hybrid cells which is the main promise of cellular genetics toward new understanding of cellular mechanisms.

B) Individual Project Descriptions:

- 1) Hybrids between dividing and non-dividing cells: fibroblast by neuron and fibroblast by heart cell hybrids.

Throughout the study of cellular genetics using hybrid cell strains much attention has been paid to the physiology of hybrids during the process of chromosome loss and rather little effort has been devoted to looking at "exconjugants", i.e., one parent cell returned after loss of all of the chromosomes of the original hybrid partner. Much of our effort with hybrid cells has addressed just this question of "exconjugant" cells.

Last year's annual report discussed our long-range attempt to obtain replicating cell strains from non-dividing cell types by hybridization. (This project was originally undertaken in collaboration with Dr. John D. Minna, Laboratory of Biochemical Genetics, NHLI.) Briefly, we found that such hybrids do divide but that in those cases studied, neurons and heart cells, the hybrids did not display the differentiated properties we were looking for. Such "extinction" of differentiated function is typical of hybrids between differentiated and non-differentiated cells. Since cases are now known where extinction does not occur, we may anticipate that some neuronal functions and some cardiac functions will ultimately be identified in our hybrids. We sought to overcome this difficulty in our experimental design by hybridizing rodent neurons with human fibroblasts; the preferential loss of human chromosomes from human-mouse hybrids was a well established principle of cell hybridology. It was anticipated that as the mouse neuron by human fibroblast hybrid strains lost human chromosomes during prolonged propagation of the hybrid that the cells should become progressively more and more neuron-like and that they would retain only those human chromosomes needed to defeat the neuron's growth control mechanism (potentially valuable information relevant to understanding malignancies derived from normal non-dividing cell types). To our surprise, however, 90% or more of our hybrid strains proved to be "reverse segregants" i.e., lost rodent chromosomes and preserved the human fibroblast's set. We made a large number of hybrids, so that the approximately 30 strains which were "normal segregants" and did lose human chromosomes may suffice for our original purposes. However, our surprising finding of a general method to produce reverse segregant rodent-human hybrid cells has sufficient importance that we must first proceed with establishing this point before we can embark on our proposed analysis of neuronal and cardiac properties of segregated hybrids.

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It has turned out that hybrids made between any freshly isolated cell type (adult or embryonic) of rodents (mouse, rat, hamster, Chinese hamster) and human fibroblast-like lines (D98 AH2 or WI 18, VA-2) will have a very high (about 90%) probability of showing reverse segregation. From the point of view of cellular genetics the most studied of these interspecific combinations is the human-mouse hybrid, so for the purposes of this summary we shall consider only the human-mouse system.

One useful application of human-mouse hybrids has been the association of one or more specific human genes with morphologically identifiable human chromosomes. This has been accomplished by noting the correlation between the presence or absence of a human function (usually an enzyme activity) in hybrids which have lost human chromosomes. By looking at enough of these examples it is possible to associate the human function unequivocally with a particular human chromosome. Of course, with reverse segregant hybrids we are now in a position to do the same thing for the linkage groups of the mouse, which is not only the most thoroughly characterized genetically of the mammals but also more useful than humans as experimental material in immunology and tumor biology. Cell lines of this kind will have valuable application to study of the host range specificity of different viruses, particularly those viruses permissive in mouse cells and not so in human cells. It is also now possible to look at the effects of partial complements of mouse chromosomes on a full set of normal human chromosomes so that we can examine the results of this kind of genetic modification of intact human cells.

Two new discoveries made this year promise further usefulness of this series of reverse segregant hybrids.

a) Several of the mouse brain by human fibroblast hybrids which have lost mouse chromosomes altogether (and have no detectable mouse DNA) have resulted in a population of exconjugant "human" cells which have fewer chromosomes than the parental human cell had. The transformed human parent, VA-2, had a mode of 77 chromosomes when the hybrids were made. Early in the hybrid strain's lifetime most of the metaphases had human chromosome counts in the fifties - none were found at fewer than 40. After all the mouse chromosomes were lost (about 18 months in culture) "human" cells were found with only 37 human chromosomes. These populations were cloned and substrains characterized. Since one of the chromosomes (G-22) was trisomic and since no Y and only one X chromosome is present it is probable that this line is monosomic for as many as 9 autosomes and the X! The picture is somewhat complicated by the fact that several of the substrains which have been karyotyped possess one or two translocation chromosomes - one involving a B and D group chromosome and the other a part of an X and another probably C group chromosome. More careful karyology must be done, but at this time it appears that chromosomes A3,B7,C10,C11,D14,D15,E16,E18 are monosomic. Human cell lines with extensive monosomy are potentially of very great importance in

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generating human mutant cell lines and in producing more simplified hybrid lines. We are currently trying to compare the frequency of occurrence of auxotrophic mutants and Ouabain resistant mutants in the monosomic lines with that of the parent VA-2 line (77 chromosomes) and of endoreduplicated sublines of the monosomic lines themselves. We are asking whether the cytological monosomy of these cells has the predicted consequences for mutation.

b) Another potentially valuable observation has been made with the reverse segregant hybrids. The largest mouse and rat chromosomes #1 and 2 are seen in the usual human-mouse and human-rat hybrids (which are losing human chromosomes) to be as long as or longer than the longest human chromosomes, A1,2,3. However, in reverse segregant hybrids which are losing rodent chromosomes, the remaining rodent chromosomes #1 and 2 are invariably about one-half the length of the human A group chromosomes regardless of the state of contraction of the particular metaphase examined. These shortened chromosomes nevertheless contain a normal QM banding pattern. We are now attempting to document this observation fully from the materials already at hand. Any mechanism which alters the physical state of the chromosomes in a regular way should be investigated further whether it be Lyonization or the chromosome shrinkage we have seen. It may prove to be an interesting lead into the mechanism by which the chromosomes of one species are lost from heterospecific hybrids.

2) The natural history of mitochondrial DNA in hybrid cells: the beginning of the genetic analysis of a human cytoplasmic organelle.

We have used an RNA-DNA molecular hybridization assay developed by Dr. Igor B. Dawid and applied by him and Dr. Ivan Horak to identify species specific nucleotide sequences of mitochondrial DNA (mt-DNA) in hybrid cell strains. Employing our unique series of human-mouse and human-rat hybrid cells we outlined in last year's annual report how it had become possible to demonstrate 1) that mt-DNA of both rodent and of man could be easily detected in these hybrid cells, and 2) that both species' mt-DNA replicated in the same cell for many cell generations - in some cases for at least 150 cell generations. Since that time many more analyses have been carried out and have finally proven what then we only suspected: human and mouse mt-DNA are not only capable of forming hybrid mt-DNA molecules in hybrid cells but apparently frequently indulge in this unique form of genetic recombination. Furthermore, in still another unique series of hybrid cells (made between mouse brain cells and a transformed human cell line) we have detected several strains which apparently contain exclusively hybrid or "recombinant" mt-DNA. It is certain that they contain a large majority of recombinant mt-DNA, and if it should turn out that they are in fact exclusively recombinant for all mt-DNA then we have a very valuable resource for the next step in the genetic analysis: use of these recombinant strains in further cell hybridizations which will define frequencies of recombination and perform functional tests of the recombinant mt-DNA. At the moment it is not at all clear just how

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in the apparent absence of a selective system (other than replication) a single form of recombinant mt-DNA could come to predominate in cells which normally contain about 1000 copies of the mt-DNA per cell. Clearly there is a selective advantage of this recombinant mt-DNA, and we may be observing an important adaptive change in the mt-DNA mediated by genetic recombination. Genetic transactions at whatever level are bound to be important in our understanding of cellular mechanisms in all cells, both normal and malignant.

It appears that we may now be able to undertake a complete genetic analysis of the mt-DNA of mammalian cells. Part of the difficulty in undertaking such an analysis in the past has been the lack of genetic markers or mutants in the mt-DNA itself. The insertion of detectable nucleotide sequences from the mouse into the human mt-DNA which we have found, is the formal equivalent of such a marker. We propose to do genetics, not by looking at the gene products alone, but by detecting the specific nucleotide sequences directly. Certain functional tests can be carried out in addition. Mitochondrial ribosomal RNA and several mitochondrial tRNAs can be assayed for species of origin, and the finding of these immediate gene products in cells bearing recombinant mitochondria will provide evidence of transcription and function in recombinant mt-DNAs as well as helping to identify which specific regions of mt-DNA may be involved in the recombination.

3) Genetic heterogeneity and complementation in fibroblasts from patients with xeroderma pigmentosum.

Patients with an inherited (autosomal recessive) disease condition known as Xeroderma pigmentosum (XP) have an acute sun sensitivity and usually die of metastatic disease probably related to the very high incidence of tumors arising in the sun-exposed areas of the skin. Five years ago Clever showed that these patients' skin fibroblasts were deficient in UV-induced ³H-thymidine (TdR) incorporation (unscheduled, DNA repair synthesis). As a result of other work showing that the chromatid breakage or X-ray-type repair system was normal in these patients, and by analogy with repair synthesis as studied in mutants of E. coli, an endonuclease defect has been widely inferred as the candidate for the fundamental defect in XP. Considerable interest has attached to this disease because of the possible link between the DNA repair defect and probable resultant mutations with the extraordinarily high incidence of cancer.

One year ago Bootsma and colleagues in Holland reported finding that fibroblasts from patients with a more severe form of the disease (involving neurological defects as well as the classical symptoms, DeSanctis Caccione syndrome) could complement or show fully restored TdR incorporation after exposure to UV light when they were fused with fibroblasts from patients with the more classical form of the disease. The complementation is mutual, i.e., both nuclei in a heterokaryon show restored repair synthesis. They

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concluded that there were at least two different forms of the disease involving separate functions capable of complementation. Inspired by the work of Bootsma's group, Dr. Jay Robbins and Dr. Kenneth Kraemer suggested to us that we undertake similar studies on the cells of patients being studied in the Dermatology Branch, NCI.

After developing a simplified technique for making these heterokaryon assays and doing many of them, we have found that a series of 13 XP patients fell into four distinct complementation groups. The cells from nine of these patients (at least one from each of the four complementation groups) have been fused in all possible combinations and analyzed by grain counting for TdR incorporation in mononucleate and binucleate cells. It turns out that the separate complementation groups are each typified by their own level of DNA repair rates rather than by their clinical manifestations (DeSanctis Caccione individuals have been found in two different complementation groups).

In principle, this kind of complementation could provide the basis for the production of mononucleate, replicating hybrid cell strains. XP patients in the two complementation groups with the lowest repair rates are 10^4 to 10^5 fold more sensitive to UV-killing than are the cells of normal donors. If the cells of patients from two different complementation groups are mixed and fused with Sendai virus, the resultant mixture of unfused parental cells, homokaryons and heterokaryons may be irradiated and a dose found which will kill all of the parental cells and homokaryons, leaving alive only those heterokaryons which have restored repair synthesis - and presumably great UV survival. We have done a few preliminary experiments like this and have found some UV-surviving colonies which have appeared only in plates in which fusion with virus was done and not in plates where parental strains were mixed but no virus was added. These strains appear to be tetraploid, but no satisfactory chromosome preparations have yet been made. The "crosses" were performed between a male and a female line so that the putative hybrids might be identified by the complement 92 + XXXY as well as by their restored level of DNA repair synthesis. If these strains prove to be hybrid then they will be the first human diploid x diploid cross to be produced and the cells will be interesting to study in their own right.

We will try to achieve these goals: 1) determine UV-survival curves for the cells of various XP patients, 2) measure mutation and "transformation" frequencies in XP cells and their hybrids, 3) make practical uses of the finding of several complementation groups in XP cells by attempting to carry the in vivo complementation tests to an in vitro system using isolated whole nuclei and hopefully thereby to establish an in vitro assay which can serve as a guide for the purification of the different active cytoplasmic extracts; ultimately such work could lead to purification of the factors involved in XP DNA repair, 4) in conjunction with other studies on mt-DNA and applying the same technology, we should be able to explore the extent to which UV damage in mt-DNA can be repaired in XP patients and in normal control fibroblasts.

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4) Antigens specific to certain cells of the rodent central nervous system produced by normally segregating rodent neuron by human fibroblast hybrids.

In collaboration with a graduate student, Mr. G. David Trisler, we are examining the antigenic constitution of the thirty normally segregating hybrid strains made by fusing embryonic mouse or rat CNS cells with the human fibroblast line WI 18, VA-2. As noted in project #1 above, we have not yet established any obvious neural properties of these cell strains which have lost different numbers of human chromosomes, from one or two lost (that is nearly complete hybrids) down to those strains containing only 5-8 human chromosomes plus the complete diploid or tetraploid rodent set. The concept of the experiment is simple, but in practice it may be hard to achieve. Hybrid strains are cloned and grown up to 10^7 cells. These are emulsified in Freund's complete adjuvant and rabbits are inoculated and booster doses are given. Sera are harvested and tested for activity against the original hybrid strain by indirect immunofluorescence (they are invariably positive). The antisera are now cross adsorbed to both live and fixed human parental cells (VA-2) until they no longer react. Then the sera are cross adsorbed to the non-neural tissues of embryonic rats and mice as well as to acetone powders of liver. It is hoped that what activity remains will be specific to the central nervous system of the rat or mouse. The cross adsorbed sera are again tested against live as well as fixed cells of the original hybrid cell strain which was used in the immunization (visualized by indirect immunofluorescence with FITC conjugated goat anti rabbit IgG). Parts of the embryonic nervous system are dissected and are treated as whole mounts and/or frozen sections are made which are treated with the cross adsorbed antisera (control sections and whole mounts are treated with cross adsorbed control normal rabbit sera). The treated tissues are rinsed thoroughly and reacted with the FITC-labelled goat anti-rabbit IgG. We hoped to find fluorescent label localized in those portions of the nervous system which contain antigens which were being expressed in the segregated hybrid cells, i.e. try to visualize the cell type which was the parent in the original hybridization.

Our first experiments have shown several anti-hybrid anti-sera which appear to fulfill this goal. We have evidence that specific groups of large neurons in the spinal cord (dorsal part) are reacting with the anti-sera made against spinal cord by fibroblast hybrids, whereas neurons right next to them and intermingled with them are dark. Of course this preliminary work must be repeated and extended but at this time it suggests to us that a most fruitful study of neuron-specific antigens may be achieved using reduced neuron by fibroblast hybrids to generate enough progeny from a single neuron to permit immunization and subsequent immunological analysis.

Serial No. NCI-5552

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Publications:

Wiebel, F. J., Gelboin, H. V., and Coon, H. G.: Regulation of aryl hydrocarbon hydroxylase in intraspecific hybrids of human, mouse and hamster cells. Proc. Nat. Acad. Sci. (USA) 69: 3580-3584, 1972.

Coon, H. G., Horak, I., and Dawid, I. B.: Propagation of both parental mitochondrial DNAs in Rat-Human and Mouse-Human hybrid cells. J. Mol. Biol. (in press).

Nyormoi, O., and Coon, H. G., and Sinclair, J. H.: Proliferating hybrid cells formed between rat spermatids and an established line of mouse fibroblasts. Journal of Cell Science (Cambridge Univ. Press). (In press)

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5552

Title: Production and analysis of hybrid somatic cells: their application to some problems of importance in biology and medicine

Principal Investigator: Hayden G. Coon

Section: Office of the Chief

Lab/Branch: Laboratory of Cell Biology, DCBD

Goal: Learn to manipulate the various genetic materials of vertebrate cells, to perform such manipulations and analyze the results of them.

Approach: A) Mononucleate hybrid cell strains are produced between defined cell types using killed Sendai virus to achieve cell fusion. Hybrid cell strains have been made between cells which do not divide in culture (eg. neurons cardiac muscle) and cell strains which do divide (fibroblasts). The specific chromosomes which enable a non-dividing cell to enter and maintain cell division are being analyzed. The process of chromosome elimination or segregation is being studied in hybrids made in different ways. Some of the human-mouse hybrids which have lost all the mouse chromosomes have also lost some human chromosomes and now are monosomic for 7 or 8 human chromosomes. These cells are being studied for production of auxotrophic mutants. B) Mitochondrial DNA (mt-DNA) has been studied in human-mouse and human-rat hybrid cells and their substrains. We are using species specific mt-DNA nucleotide sequences as the genetic markers followed in hybrid cell crosses. C) Genetic heterogeneity among Xeroderma pigmentosum (XP) patients is being studied by fusing skin fibroblasts of different patients and assessing DNA repair in the heterokaryons. Since cells from persons in different complementation groups show restored DNA repair, UV light may prove a method for selecting mononucleate hybrid strains from these diploid human cells.

Progress: 1) Human-mouse hybrid cells usually lose human chromosomes. A general method has been found which produces human-mouse hybrids which lose mouse chromosomes. Mouse linkage groups can now be identified. The effects of partial mouse chromosome complements on human cells can be observed. Doubly segregated hybrid cells have resulted in human cells monosomic for as many as 8 chromosomes. 2) A method for achieving a genetic analysis of mammalian mt-DNA has been found. We have shown that both human and mouse mt-DNA can replicate in the same cytoplasm and have found the first evidence that higher eukaryotes' mt-DNA is capable of recombination. In hybrid cells, interspecific recombination is a frequent event and implies that adaptive changes may accumulate in normal cells' mt-DNA by recombination. 3) A third and fourth complementation group have been described among XP patients.

Significance for Cancer Research (NCP Objective 3 Approach 1)
Factors influencing growth control in non-dividing cell types are directly relevant. Human cells containing a few rodent chromosomes should prove useful in studying tumor viruses, both murine and human. Extensively monosomic human cell strains should assist in evaluating the mutagenic potential of carcinogens. The existence of 4 complementation groups in XP should provide handles for biochemical identification and isolation of the specific molecular defect(s) present in this cancer-prone condition. The discovery of a new level of genetic interactions in mammalian cells is (mt-DNA) important for cancer research.

Level of Effort: Professional Man-Years: One

Serial No. NCI-5562

1. Laboratory of Cell Biology
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Isolation and solubilization of SV-40
tumor-associated transplantation antigens.

Previous Serial Number: None

Principal Investigator: Mark S. Drapkin, M.D.

Other Investigators: Dr. L. W. Law
Dr. E. Appella

Man Years:

Total:	2
Professional:	1.5
Other:	0.5

Project Description:

1. Crude membranes (CM) were prepared from SV-40 transformed non-virus producing BALB/c mouse cells (MKSA cells) by nitrogen decompression. One or two subcutaneous injections of 0.3 to 1.0 mgm of CM into syngeneic mice yielded a high degree of tumor-specific protection against subsequent viable MKSA cell challenge. Crude soluble material (CS), obtained by limited papain digestion of CM, was also immunogenic. CS yielded three distinct peaks by G-150 sephadex chromatography; preliminary data suggest that one of these contains immunogenic material.

These data suggest that tumor-associated transplantation antigens are somewhat similar to histocompatibility antigens previously isolated by similar means.

2. ⁵¹Cr-labelled target cells were used for in vitro study of tumor antigen expression. Data indicate that SV-40 transplantation antigens isolated as described above are not identical to SV-40 antigens eliciting cytotoxic antibody production in hyperimmune animals. This dichotomy between in vivo and in vitro antigen expression demands caution in interpreting the latter.

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 5562

Title: Isolation and solubilization of SV-40 tumor-associated transplantation antigens.

Principal Investigator: Mark S. Drapkin, M.D.

Section: Office of the Chief

Lab/Branch: Laboratory of Cell Biology, DCBD, NCI

Goal: To isolate and characterize immunogenic subcellular fractions of non-virus producing tumor cells.

Approach: Crude membrane (CM) was isolated from SV-40 transformed non-virus producing BALB/c mouse cells (MKSA cells) by nitrogen decompression and by sonication. Crude soluble material (CS) was obtained by limited papain digestion of CM; CS was chromatographed on G-150 Sephadex.

The specific immunogenicity of CM, CS and CS fractions was investigated using standard immunization-challenge protocols. A standard complement-mediated cytotoxic inhibition assay with ^{51}Cr -labelled target cells was employed for in vitro studies of antigen expression.

Progress: Data indicate that CM and CS, obtained as described above, are highly immunogenic in vivo in syngeneic mice. One CS fraction may also be active in vivo. In vitro data demonstrate that these tumor-associated transplantation antigens are not identical with SV-40 antigens eliciting cytotoxic antibody in hyperimmunized animals.

Significance for Cancer Research (NCP Objective 3 Approach 5)

The isolation of virus-free tumor cell membrane antigens capable of immunizing syngeneic animals raises the possibility that such antigens may provide a rational tool for immunotherapy. The demonstration of a dichotomy between in vivo and in vitro antigen expression demands caution in interpreting all in vitro data.

Level of Effort: Professional Man-Years: 1.5

Serial No. NCI-5557

1. Laboratory of Cell Biology
2. Cellular and Tumor Immunology
Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Immune Response to Tumor Specific and Other Cell
Surface Antigens

Previous Serial Number: Same

Principal Investigator: Ronald B. Herberman, M.D.

Other Investigators: Chou-Chik Ting, M.D., Devron Char, M.D.,
Moshe Glaser, Ph.D.

Cooperating Units: Hematology and Supportive Care Branch, NCI;
Viral Leukemia and Lymphoma Branch, NCI;
Surgery Branch, NCI; Laboratory of Microbiology,
NIDR; George Washington University, Contracts
PH 69-2176 and 72-3251; Georgetown University
School of Medicine; Litton Bionetics, Inc.,
Contracts PH 69-2160, 72-3227 and 72-3878.

Man Years:

Total: 10-1/2

Professional: 5-1/2

Other: 5

Project Description:

Objectives: The objectives of this project are: 1) to detect tumor specific and tumor associated antigens and quantitative changes in normal antigens on malignant cells; 2) to detect antibodies and cellular immune reactions to tumor antigens; 3) to evaluate the diagnostic, prognostic and therapeutic significance of humoral and cellular immune response to tumor antigens; 4) to develop and evaluate immunodiagnostic assays for human cancer; 5) to measure general immunological competence of cancer patients; 6) to detect delayed hypersensitivity reactions and other immunological reactions to human tumor antigens; and 7) to isolate and characterize tumor antigens and other cell surface antigens.

Methods Employed: Sera and cells are obtained from patients and control subjects. A variety of normal and tumor cell lines are maintained in tissue culture. Inbred strains of mice and

rats, and transplantable tumors induced by viral, chemical or unknown agents are used in model systems. Antigen-antibody reactions are measured by complement-dependent cytotoxicity reactions and by an indirect ^{125}I -labeled antibody technique. Cellular immune reactions are measured by skin tests, mixed lymphocyte cultures, migration inhibition, and lymphocyte-mediated cytotoxicity assay. Human and animal tumor and normal cell membrane and soluble fractions are prepared for antigenic and biochemical studies. Tumor cells and normal lymphocytes are tested for membrane receptors by immunofluorescence and by rosette formation with sheep erythrocytes.

Major Findings:

I. Tumor Immunity in Animal Models:

a. The cellular and humoral immune response to Gross virus-induced cell surface antigens has been extensively studied. Direct cell-mediated cytotoxic reactivity was found 7-21 days after inoculation of a transplantable Gross leukemia into syngeneic W/Fu rats. Cells from rats inoculated 40 or more days before had little or no direct activity but upon pre-incubation at 37°C , underwent in vitro activation. Studies on the mechanism of both forms of cytotoxicity have shown that the directly active cells are T cells, inhibited by pretreatment with a specific anti-rat T cell serum and not inhibited by removal of B cells on adherence columns or on columns containing complement-coated erythrocytes. These cells are also inhibited by cytochalasin B and cyclic AMP. In contrast the activity of the in vitro activated cells was not affected by the anti-T cell serum or by cytochalasin B, but was reduced by removal of B cells.

b. Similar studies have been performed on cell-mediated cytotoxic reactivity after inoculation of mice with Moloney sarcoma virus (MSV). Directly cytotoxic lymphocytes, both at the peak of reactivity (14 days after inoculation) and later (up to 70 days) were inhibited by treatment with anti-theta serum and also by cytochalasin B and cyclic AMP.

c. An inhibition assay has been developed in our laboratory for studying the specificity of the cellular cytotoxic reactions. The specificity of the cellular reactions against Gross leukemias in rats and against MSV in mice have been found to be quite different from the specificities of antibodies. In both systems, the detected antigens are almost entirely species specific and may be related to antigens induced by the endogenous C-type viruses of each species. Antigens have been detected on cells containing virus genetic information, but not producing any infectious virus. This provides strong evidence that the detected antigens are distinct from virus envelope antigens. In

the MSV system, there was also a partial strain restriction to the detected antigens. C57BL/6 mice recognized an antigen present on C57BL/6 tumor cells but absent on similar cells of BALB/c origin.

d. Naturally occurring cell-mediated immunity in both mice and rats, reactive with virus-induced leukemic cells has been found. This cytotoxic reactivity appeared at 3-4 weeks of age, and rose to a peak at 6-8 weeks. The specificity of the natural reactivity was similar to, but not identical with, that induced by immunization with virus or tumor cells. The mechanism for natural sensitization remains unclear, but environmental factors appeared to be important. Germfree mice and rats had significantly less reactivity than age-matched animals raised under conventional conditions.

e. Activation of cytotoxic reactivity of lymphocytes by phytohemagglutinin (PHA) and endotoxin has been studied. Activated rat cells were able to lyse a syngeneic leukemic target cell. The PHA activated cells appeared to be T cells, sensitive to the specific anti-T cell serum and not affected by passage through a column containing complement-coated erythrocytes (EAC). The endotoxin activated cells may be "null" lymphocytes, since they were not inhibited by the anti-T cell serum nor by the EAC column.

f. Stimulation of lymphocytes by syngeneic tumor cells or extracts of tumor cells has been studied. Lymphocytes from rats immune to a syngeneic Gross virus-induced leukemia were stimulated by X-irradiated tumor cells and even better by a hypertonic salt extract of the tumor cells.

g. Cell surface receptors for complement component C3 and for immunoglobulins have been shown to be present on bone marrow derived lymphocytes (B cells) and absent on thymus derived cells (T cells). A large number of rat leukemias and lymphomas were tested for these receptors, as markers for the origin of the tumor cells. One tumor was found to contain surface immunoglobulin but lacked the receptor for complement. This cell line has proved very useful in absorbing an anti-rat thymus cell serum, rendering it specific for T cells.

h. The specificity of the antibodies induced in rats and mice by immunization with Gross virus-induced leukemias. Rather than a single G cell surface antigen, antibodies could be identified which reacted with at least four different surface antigens: Gross cell surface antigens (GCSAa, GCSAb, GCSAc, and GIX).

II. Tumor Immunity in Man:

a. Patients with neoplastic diseases have been shown to have delayed hypersensitivity reactions to extracts of tumor cells. In acute leukemia, delayed skin reactivity to crude membrane extracts of autologous and allogeneic blast cells has correlated with remission status of the patients. There has also been a correlation with type of drug therapy and with prolonged remission. In contrast, skin reactivity to recall antigens has been found to be intact in most patients, even during relapse.

b. Patients with acute leukemia and with lymphoma have also been found to react with membrane extracts of lymphoid cell lines derived from patients with Burkitt's tumor and not with extracts from a normal lymphoid cell line. Patients with carcinomas had no reactivity to these materials. Reactivity of the leukemia patients correlated with remission status. The nature of the antigen causing these reactions has not been determined, but in lymphoma patients, reactivity correlated with elevated antibody titers to EB virus associated antigens.

c. Antigens on acute myelogenous leukemia cells have been solubilized by low frequency sonication, in collaboration with Dr. A. Hollinshead (George Washington University), after chromatography of the sonicate on Sephadex G-200, a skin reactive fraction was found, which could be further purified by DEAE cellulose and acrylamide gel electrophoresis. In contrast, no positive reactions were obtained with the unfractionated sonicate; it has been found that this contains a blocking factor, which inhibits skin reactivity to tumor extracts and not to recall antigens.

d. Further studies have been performed with the skin reactive antigen in intestinal cancer. This antigen has been found to be a contaminant of CEA preparations from several laboratories, yet is distinct from the CEA of Gold and from the NCA of Von Kleist.

e. Soluble skin reactive antigens from carcinomas of the breast and lung, and from melanoma, have also been studied. In each of these diseases, there is reactivity to an antigen present in normal tissue extracts as well as in tumor extracts.

f. The lymphocytes of almost all normal individuals have been found to have cytotoxic reactivity against human lymphoid cell lines in the ^{51}Cr release assay. The detected antigen appears to be unrelated to EB virus, to mycoplasma, or to fetal bovine serum. Patients with cancer or immunosuppression have decreased or no activity, and this assay may prove useful in monitoring the general competence of lymphocytes to produce

cytotoxicity. This reactivity has been correlated with ability of lymphocytes to be stimulated by PHA.

g. Assays for the relative proportions of T and B lymphocytes in the peripheral blood of cancer patients and other individuals have been performed, by measuring the binding of sheep erythrocytes (E) and complement-coated erythrocytes (EAC). Many patients with cancer, particularly those with advanced disease, have been found to have decreased percentages of E-binding cells and increased numbers of EAC-binding cells. Patients with Hodgkin's disease have been most interesting, with both types of cells decreased. Pleural effusions of cancer patients have been found to contain high proportions of E-binding cells, at a time when peripheral E-binding cells are low.

h. Extensive in vitro assays are being performed to measure cellular immune reactions to human tumor associated antigens. A leukocyte migration assay has been developed and antigens like PPD and SKSD have been shown to reliably cause inhibition of migration of the leukocytes of skin test positive individuals. The leukocytes of cancer patients have been inhibited by hypertonic salt extracts of autologous or allogeneic tumors of the same histological type. Some of these assays have also been found to cause stimulation of the lymphocytes of cancer patients, to incorporate increased amounts of ³H-thymidine.

Significance to Biomedical Research and the Program of the Institute:

Detection of tumor antigens and of immune reactions to tumor specific antigens is essential for progress in tumor immunology. The sensitive assays for humoral and cell-mediated immunity which have been developed in the laboratory have already proved useful in studying the immune response to animal and human tumors.

The ability of our laboratory to perform multiple assays of humoral and cell-mediated immunity to tumor antigens should allow detailed dissection of the role of the immune response in limiting or preventing tumor growth. These assays may also be useful in immunodiagnosis of cancer and in measuring changes in immunity, in response to chemotherapy or immunotherapy.

The available immunological techniques have also allowed progress in the solubilization and isolation of tumor-associated cell surface antigens. Such studies will permit a comparison of the physicochemical nature of tumor antigens and normal histocompatibility antigens and may provide material for active immunization of animals and tumor patients against their tumors.

Proposed Course of Project:

Studies are in progress to further study the immune responses of experimental animals and of patients to tumor associated antigens.

In experimental animals, further studies will be performed on the nature of the cells involved in various immune fractions and on their mechanism of action. The interrelationships between different assays will be extensively studied. The effects of sera in causing blocking or increased activity in cell-mediated assays will be examined, and the responsible factors and their specificity studied. Cells and sera shown to be reactive in the in vitro assays will be used in immunotherapy and immunoprophylaxis experiments.

Studies of human tumor associated antigens and immune reactions to them will be continued. Skin reactivity to tumor antigens and in vitro assays will be used to monitor the response of patients with acute lymphocyte leukemia, melanoma and sarcoma to immunotherapy with BCG or with tumor cells. Separated soluble skin reactive antigens will be used for immunization of heterologous species, in an attempt to produce antisera which might have diagnostic usefulness. The soluble antigens are also being tested for blocking of in vitro lymphocyte functions and for lymphocyte stimulation.

The studies on the proportion of T and B cells in the peripheral blood of cancer patients will be continued, to determine their usefulness for diagnosis and for prognosis. The correlation between these results and activity in other assays of cell-mediated immunity will be studied. These techniques will also be used to determine the type of cells which are active in various in vitro assays of cell-mediated immunity.

Several blood markers have been identified which may have diagnostic usefulness: CEA, alphafetoprotein, Regan enzyme, and various hormones. Studies are being set up to perform several of these assays on the same serum specimens, to determine the possible value of performing multiple assays for detection of cancer.

Honors and Awards:

Ronald B. Herberman

Appointed Chairman, Committee for Immunodiagnosis of Cancer

Editorial Board, Journal of Immunology

Editorial Advisory Board, Cancer Research

Publications:

1. Ortiz de Landazuri, M., and Herberman, R.B.: Immune response to Gross virus-induced lymphoma. III. Characteristics of the cellular immune response. J. Nat. Cancer Inst., 49: 147-154, 1972.
2. Engelstein, J.M., Herberman, R.B., and Waltman, S.R.: Protection of penetrating corneal allografts from immune rejection. Amer. J. Ophthal., 74:311-315, 1972.
3. Ting, C.C., Lavrin, D.H., and Herberman, R.B.: Antibodies to fetal antigens. Proc. Second Conf. and Workshop on Embryonic and Fetal Antigens in Cancer, N.G. Anderson and J.H. Coggin, Jr. (Eds.), pp. 111-116, 1972.
4. Rosenberg, E.B., Herberman, R.B., Levine, P.H., Halterman, R.H., McCoy, J.L., and Wunderlich, J.R.: Lymphocyte cytotoxicity reactions to leukemia-associated antigens in identical twins. Int. J. Cancer, 9:648-658, 1972.
5. Herberman, R.B.: Immunological aspects observed in childhood malignancies; rationale for immunotherapy of these tumors. In Clinical Pediatric Oncology, M.M. Vuksanovic (Ed.), Futura Publishing Co., Inc., Mount Kisco, N.Y., 1972, pp. 15-23.
6. Ortiz de Landazuri, M., and Herberman, R.B.: Specificity of cellular immune reactivity to virus-induced tumors. Nature-New Biology, 238:18-19, 1972.
7. Ting, C.C., Lavrin, D.H., Shiu, G., and Herberman, R.B.: Expression of fetal antigens in tumor cells. Proc. Nat. Acad. Sci., 69:1664-1668, 1972.
8. Shevach, E.M., Herberman, R., Frank, M.M., and Green, I.: Receptors for complement and immunoglobulin on human leukemic cells and human lymphoblastoid cell lines. J. Clin. Invest., 51:1933-1938, 1972.
9. Hollinshead, A., McWright, C., Alford, T., Glew, D., Gold, P., and Herberman, R.B.: Separation of skin-reactive intestinal cancer antigen from the carcinoembryonic antigen of Gold. Science, 177:887-889, 1972.
10. Herberman, R.B., and Aoki, T.: Immune and natural antibodies to syngeneic murine plasma cell tumors. J. Exp. Med., 136:94-111, 1972.

11. Leventhal, B.G., Halterman, R.H., Rosenberg, E.B., and Herberman, R.B.: Immune reactivity of leukemia patients to autologous blast cells. *Cancer Res.*, 32:1820-1825, 1972.
12. Levine, P.H., Herberman, R.B., Rosenberg, E.B., McClure, P.D., Roland, A., Pienta, R.J., and Ting, R.C.Y.: Acute leukemia in identical twins: Search for viral and leukemia associated antigens. *J. Nat. Cancer Inst.*, 49:943-952, 1972.
13. Ortiz de Landazuri, M., and Herberman, R.B.: In vitro activation of cellular immune response to Gross virus-induced lymphoma. *J. Exp. Med.*, 136:969-983, 1972.
14. Aoki, T., Herberman, R.B., Johnson, P.A., Liu, M., and Sturm, M.M.: Wild-type Gross leukemia virus: Classification of soluble antigens (GSAs). *J. Virology*, 10:1208-1219, 1972.
15. Green, I., Shevach, E.M., Stobo, J., Frank, M., and Herberman, R.: B cell, T cell and monocyte origin of the guinea pig L₂C leukemia, mouse lymphomas and human leukemias and lymphoblastic cell lines. In *Microenvironmental Aspects of Immunity*, B.D. Jankovic and K. Isakovic (Eds.), Plenum Publishing Corp., New York, New York, 1972, pp. 491-498.
16. Herberman, R.B.: In vivo and in vitro assays of cellular immunity to human tumor antigens. *Fed. Proc.*, 32:160-164, 1973.
17. Herberman, R.B., Aoki, T., and Nunn, M.E.: Solubilization of G(Gross) antigens on the surface of G leukemia cells. *J. Nat. Cancer Inst.*, 50:481-490, 1973.
18. Herberman, R.B.: Cellular immunity to human tumor associated antigens. *Israel J. Med. Sci.*, in press.
19. Hollinshead, A.C., and Herberman, R.B.: Separation of the major histocompatibility antigens from other antigens present on human leukemic and white blood cell membranes. *Proc. Fifth International Symposium on Comparative Leukemia Research*, in press.
20. Herberman, R.B., and Rosenberg, E.B.: Cellular cytotoxicity reactions to human leukemia associated antigens. *Proc. Fifth International Symposium on Comparative Leukemia Research*, in press.

21. Herberman, R.B., Rosenberg, E.B., Halterman, R.H., McCoy, J.L., and Leventhal, B.G.: Cellular immune reactions to human leukemia. NCI Monograph #35:259-266, 1972.
22. Herberman, R.B.: In vivo methods: Delayed hypersensitivity response toward autochthonous tumor extracts. Proc. Colloquium of the Centre National de la Recherche Scientifique on "The Investigation and Stimulation of Immunity in Cancer Patients," in press.
23. Oldham, R.K., McCoy, J.L., Plata, E.J., and Herberman, R.B.: Evaluation of a cell-mediated cytotoxicity assay utilizing ¹²⁵Iododeoxyuridine labelled tissue culture target cells. J. Nat. Cancer Inst., in press.
24. Leventhal, B.G., Halterman, R.H., Rosenberg, E.B., McCoy, J.L., and Herberman, R.B.: Lymphocyte cytotoxicity in human acute leukemia. Proc. Colloquium of the Centre National de la Recherche Scientifique on "The Investigation and Stimulation of Immunity in Cancer Patients," in press.
25. Herberman, R.B., Hollinshead, A.C., Alford, T.C., McCoy, J.L., Halterman, R.H., and Leventhal, B.G.: Delayed cutaneous hypersensitivity reactions to extracts of human tumors. J. Nat. Cancer Inst., in press.
26. Herberman, R.B.: Immunologic reactions of experimental animals to tumor associated cell surface antigens, Pathobiology Annual, in press.
27. McCoy, J.L., Herberman, R.B., Rosenberg, E.B., Donnelly, F.C., Levine, P.H., and Alford, C.: ⁵¹Chromium release assay for cell-mediated cytotoxicity of human leukemia and lymphoid tissue culture cells. NCI Monograph, in press.
28. Alford, C., Hollinshead, A.C., and Herberman, R.B.: Delayed cutaneous hypersensitivity reactions to extracts of malignant and normal human breast cells. Ann. Surg., in press.
29. Gazdar, A., Hatanaka, M., Herberman, R., Russel, E., and Ikawa, Y.: Effects of dibutyryl cyclic adenosine phosphate plus theophylline on murine sarcoma virus transformed non-producer cells. Proc. Soc. Exp. Biol. Med., in press.
30. Leventhal, B.G., Lepourhiet, A., Halterman, R.H., Henderson, E.S., and Herberman, R.B.: Immunotherapy in previously treated acute lymphatic leukemia. NCI Monograph, in press.

31. Gazdar, A.F., Russell, E.K., and Herberman, R.B.: Mouse strain related differences in the biologic and immunologic responses to a murine sarcoma virus. J. Nat. Cancer Inst., in press.
32. Rosenberg, E.B., Hill, J., Ferarri, A., Herberman, R.B., Ting, C.C., Mann, D.L., and Fahey, J.: Prolonged survival of tumor allografts in mice pretreated with soluble transplantation antigens. J. Nat. Cancer Inst., in press.
33. Herberman, R.B., Lepourhiet, A., Hollinshead, A., Char, D., McCoy, J.L., and Leventhal, B.G.: Humoral and cell-mediated immunity in human acute leukemia. Proc. Twenty-Sixth Annual Symposium on Fundamental Cancer Research, in press.
34. Herberman, R.B., McCoy, J.L., and Levine, P.H.: Immunologic reactions to tumor associated antigens in Burkitt's lymphoma and other lymphomas. Proc. Workshop on Human Tumors Associated with Herpesvirus, in press.
35. Ting, C.C., Ortaldo, J.R., and Herberman, R.B.: Expression of fetal antigens and tumor specific antigens in SV40 transformed cells. I. Serological analysis of the antigenic specificities. Int. J. Cancer, in press.
36. Ting, C.C., Rodrigues, D., and Herberman, R.B.: Expression of fetal antigens and tumor specific antigens in SV40 transformed cells. II. Tumor transplantation studies. Int. J. Cancer, in press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 5557

Title: Immune Response to Tumor Specific and Other Cell Surface Antigens

Principal Investigator: Ronald B. Herberman, M.D.

Section: Cellular and Tumor Immunology Section

Lab/Branch: Laboratory of Cell Biology

Goal: To detect tumor specific and tumor associated antigens and to measure the cell-mediated and humoral immune reactions to these antigens.

Approach: Study the immune response to tumor associated antigens on virus-induced tumors of experimental animals and also to human tumors. Define the specificity of the detected antigens and the nature of the cells mediating the responses in various assays. Evaluate the diagnostic, prognostic and therapeutic significance of humoral and cellular immune response to tumor antigens. Develop and evaluate immunodiagnostic assays for human cancer. Measure general immunological competence of cancer patients. Isolate and characterize tumor antigens and other cell surface antigens.

Progress: Immune T cells have been shown to cause the direct cytotoxic effects against syngeneic Gross virus-induced rat leukemia and murine leukemias. In vitro activated cells appear to be B cells. The specificity of the antigens detected in these cell-mediated cytotoxicity reactions have been studied and found to be quite distinct from the specificities of antibodies: they are species specific and may be related to antigens of endogenous leukemia viruses. Patients with neoplastic diseases were shown to have delayed skin reactions to tumor antigens. In vitro assays of lymphocyte stimulation and migration inhibition have also been positive with tumor extracts. Cancer patients have been found to often have decreased numbers of circulating T cells and also decreased cytotoxic lymphocytes.

Significance for Cancer Research (NCP Objective^{5B} Approach⁴)

Detection of tumor antigens and of immune reactions to tumor specific antigens is essential for progress in tumor immunology. These assays may be useful in immunodiagnosis and in monitoring immunotherapy.

Level of Effort: Professional Man-Years: 5-1/2

Serial No. NCI-5560

1. Laboratory of Cell Biology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Immunologic Studies of the Mechanism of Syngeneic Rejection of an SV40 Virus Induced Tumor

Principal Investigator: Stephen Howell

Other Investigators: E. Esber (NCI)

Man Years:

Total: 1
Professional: 1

Project Description:

Evidence exists that the immune state of a tumor bearing host changes as the tumor grows. The immune state of specifically immunized and tumor bearing mice has been investigated using three in vitro tests of cell mediated immunity.

MKSA is an SV40 derived BALB/c mouse tumor which is carried in vivo in both an ascitic and solid form, and in vitro as a tissue culture line (TU5). The MKSA tumor carries a particularly strong tumor specific transplantation antigen. A single immunization with TU5 cells, which are highly immunogenic but poorly tumorigenic, is capable of protecting mice against a lethal challenge of MKSA ascites tumor. Cell mediated immunity in immunized and tumor bearing animals has been assayed using a chromium 51 release cytotoxicity test, the Winn test, and the microcytotoxicity test of Hellstrom (in collaboration with Dr. E. Esber, NCI). The results have been correlated with ability to resist tumor challenge.

Lymphocytes from immunized and tumor bearing hosts prevent the growth of tumor when injected s.c. into virgin animals together with MKSA ascites cells. The same lymphocytes produce significant inhibition of growth in the microcytotoxicity test. However, these lymphocytes do not damage tumor cells appreciably in vitro as measured by the chromium release assay, even at a time when immunized and tumor bearing animals are highly resistant to tumor challenge. Activity in the chromium release assay can be generated by in vitro incubation of the attacking cells. Experiments with tumor bearing animals indicate that these mice

Serial No. NCI-5560

are capable of resisting a lethal challenge of tumor delivered at a distant site.

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 5560

Title: Immunologic Studies of the Mechanism of Syngeneic Rejection of an SV40 Virus Induced Tumor

Principal Investigator: Stephen Howell

Section: Office of the Chief

Lab/Branch: Laboratory of Cell Biology

Goal: This study is concerned with elucidating the mechanism(s) whereby specifically immunized mice are capable of resisting the growth of a viral induced tumor.

Approach

MKSA is an SV40 derived BALB/c mouse tumor which is carried in vivo in both an ascitic and solid form, and in vitro as a tissue culture line (TU5). The MKSA tumor carries a particularly strong tumor specific transplantation antigen. A single immunization with TU5 cells, which are highly immunogenic but poorly tumorigenic, is capable of protecting mice against a lethal challenge of MKSA ascites tumor. Cell mediated immunity to the MKSA tumor in both immunized and tumor bearing animals has been measured using three in vitro assays: a chromium⁵¹ release cytotoxicity test, the Winn test, and the microcytotoxicity test of Hellstrom (in collaboration with Dr. E. Esber, NCI). The results have been correlated with the ability of mice to resist a lethal tumor challenge.

Progress:

Immune lymphocytes prevent the growth of tumor when injected s.c. into virgin mice together with MKSA ascites cells, and produce significant cytotoxicity on a microcytotoxicity test (Hellstrom). However, freshly harvested immune lymphocytes do not damage tumor cells appreciably in vitro as measured by the chromium⁵¹ release assay. Investigations in progress include several methods of treating immune cells in vitro to increase their activity in the Cr⁵¹ release assay. Experiments with animals bearing progressively growing MKSA subcutaneous tumors indicates that these mice are capable of resisting a lethal challenge of the same tumor delivered at another site, and that lymphocytes from such an animal are capable of suppressing tumor growth in a Winn test.

Significance for Cancer Research (NCP Objective 6 Approach 1): Since all human tumors are syngeneic, knowledge of exactly how syngeneic tumors are rejected is fundamental to the practical application of immunotherapy.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-3200

1. Laboratory of Cell Biology
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH

Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Factors influencing the induction and repression of primary tumors and the growth and inhibition of transplantable neoplasms. (Host and tumor cell variables)

Previous Serial Number: Same

Principal Investigator: Dr. Lloyd W. Law

Other Investigators: Dr. E. Appella (NCI-3229)
Dr. Kenneth Chang (NCI-5550)
Dr. James McCoy (Bionetics)
Dr. Jack Dean (Bionetics)
Dr. Samuel Strober (Stanford University School of Medicine, Stanford, Calif.)
Dr. Stephen Howell (NCI-5560)
Dr. Mark Drapkin (NCI-5562)
Dr. James Cohen (NCI-5561)
Dr. Kenneth Takemoto (NIAID-71J)
Dr. Orsala Pugliese - Visiting Fellow (NCI)

Man Years: Total - 7 1/2
Professional - 4
Other - 3 1/2

Project Description:

1. Studies of the biologic properties of solubilized, partially purified (by G-150 Sephadex fractionation) H-2^a antigens continue as well as attempts to solubilize and purify virus-specified tumor antigens of the transplantation type (VSTA).

Collaborative studies of H-2 antigens with Dr. E. Appella have revealed the following pertinent findings:

a) Although the biochemical methods used for solubilization and purification have shown preservation of most of the biologic functions of H-2^a such as the capacity to immunize and to achieve immunologic enhancement, immunologic tolerance as induced in neonates or in adult x-irradiated B-10D2 congenic mice is restricted to humoral tolerance. Neonates receiving 40 and 0.4 µgms of the purified F2 fraction from birth through 4 weeks are unable to produce cytotoxic, hemagglutinating and enhancing antibodies but nevertheless retain the capacity to reject B10.A skin and heart grafts.

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Neonates receiving from birth 4 μ gms of the F2 fraction were not rendered humorally tolerant.

Those mice, made humorally tolerant with soluble antigen, have an increased cell-mediated response at least as revealed by the graft versus host (GVH) reaction and in the mixed lymphocyte reaction (MLR).

In collaboration with Dr. James Cohen (NCI-5561), using an in vitro Cr^{51} release assay for lymphocytotoxicity, we have investigated the role of "blocking" factors in the sera of our humorally tolerant B10D2 mice and those made completely tolerant by transfer of lymphoid cells at birth. We have found specific "blocking" in those antisera obtained from "responder" and hyperimmunized (against H-2^a antigens) mice but not from B10D2 mice made humorally tolerant (non-responders) nor from mice with complete tolerance. The induction of specific immunologic enhancement, using Sa I, parallels the induction of "blocking" in vitro and both appear to correlate with high levels of cytotoxic antibody.

Work is in progress in an attempt to study B cell and T cell functions in our humorally tolerant B10D2 recipients receiving soluble H-2^a antigens. These studies entail reconstitution of thymectomized mice with thymus and lymph node cells from tolerant or normal B10D2 mice and reconstitution of lethally x-rayed B10D2 adults with bone marrow cells from tolerant or normal mice. B and T-cell functions in these groups will then be analyzed.

We have prepared soluble H-2 antigens from another source (B10D2 spleens) and work is in progress to tolerize B10A neonates with this material. The induction of complete tolerance through the intravenous transfer of lymphoid cells at birth in the combination B10D2 $\rightarrow$ B10A is strikingly easier to attain than in the reciprocal situation we have used as described above - and in this direction of transfer only a single strong specificity, H-2 $\cdot$ 31 is involved. It is our hope that we may achieve complete immunologic tolerance with soluble H-2^d antigen.

b) Work continues in our attempt to solubilize and purify two strong VSTA: the membrane antigen on RBL-5 leukemic cells - an RLV-specified antigen and on mKSA fibrosarcoma cells - an SV40 virus specified antigen. The following findings are pertinent:

1.) Activity of the RBL-5 antigen is specific and is detected in Cr^{51} in vitro inhibition using an F2 fraction. In vivo immunogenicity against LSTRA and FBL-3 (both sharing FMR antigens) has been achieved. Sensitive assays for the detection of infectious MuLV in our preparations have been negative thus indicating that the RBL-5 antigen is a new cellular antigen and not a viral envelope antigen.

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2) Collaborative work with Dr. Mark Drapkin (NCI-5562) continues with the mKSA antigen. Crude soluble and F2 fraction material provides a specific and clear immunogenicity against the grafting of mKSA cells.

3) The yield of active material following papain digestion is very low (in terms of protein or cytotoxic-inhibiting units) in relation to our H-2 preparations and further attempts are being used with other methods of solubilization and other sources of starting material to increase our yields of both VSTA .

2. Studies of the immune response to different tumor antigens.

a) In collaboration with Dr. K. Takemoto (NIAID-71J) we are studying the membrane antigens of three different fibrosarcomas induced in vitro by the slow viruses, Visna and Progressive pneumonia virus (PPV) of sheep. These neoplasms in A/LN and BALB/c mice used directly from tissue culture or after in vivo adaptation contain antigens that interestingly do not cross-react, in contrast to the group-specific sharing of most antigens that are virus-specified. The viral genomes are recoverable from these neoplasms through cultivation with sheep testis cells but exhaustive assays for leukemic-sarcoma viruses, SV40, polyoma and adeno-12 viruses have been consistently negative. Work continues on the biologic characterization of these antigens, the relationship of viral genome to expression and the development of in vitro assays.

b) Antigens of neoplasms induced by the RNA-containing virus, M-MSV. It has been difficult to characterize the VSTA of neoplasms induced by MuLV and MSV-pseudotype viruses since usually infectious virus is continuously released by these tumor cell populations: thus it has not been possible to distinguish between new cellular antigens and antigens of the viral membrane. Through development of a non-producer hemangiosarcoma XM-1 originally induced by M-MSV we were able to show the existence of VSTA apparently not structurally related to the virus. XM-1 has now been carried in syngeneic recipients for 100 transfers and continues to express its VSTA; occasionally though we are now able to detect infectious MSV and MuLV. It is likely that residual MLV is carried in this neoplasm and that this associated helper virus is able to rescue MSV.

Through the establishment of a series of in vitro clones we were able to gain some insight into the relationship of these viruses to the expression of VSTA. Clones H-1, H-11 H-12B, H-13 and H-14 were established and assays for

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infectious viruses, defective virus (MSV) and antigenicity (including cross-reactivity) accomplished. Clone H-12B was non-oncogenic but capable of immunizing. H-1 and H-11 were capable of immunizing and were sensitive to the rejection response of M-MSV or of other clones; neither clone was a non-producer but when established *in vivo* now produced MuLV which did not affect their immunogenicity nor their immunosensitivity. Clone H-14 though containing infectious MuLV was neither immunogenic nor immunosensitive. Cross-reactivity was observed among all the clones and the parental XM-1 line, H-14 appears to be an exception to the rule that all virus-induced tumors possess group-specific VSTA. Again it appears that we are studying new cellular antigens specified by MSV and not related to infectious virus. (Collaborative studies with Dr. K. Chang, NCI-5550). We have initiated collaborative studies with Dr. T. Aoki (NCI-4864) to look for the virus-associated (MSV) cell-surface antigens he has described by immunoelectron microscopy. This is a newly described antigen found in non-producer neoplasms induced by MSV and appears to be distinct, at least serologically, from those previously described.

c) In collaboration with Dr. Stephen Howell (NCI-5560) the mechanism of inhibition of tumor growth *in vivo* and *in vitro* (syngeneic system-BALB/c mice) is being studied. A tissue culture-adapted line of an SV-40 fibrosarcoma, mKSA, is strikingly immunogenic but non-oncogenic; 1×10^5 to 1×10^6 cells are capable of immunizing against lethal doses of the parental ascites line. Sensitized lymphocytes, so obtained, (microcyto-toxicity test) and more recently in the Cr⁵¹ release assay. This experimental system therefore allows us to determine the meaning of *in vitro* assays in terms of *in vivo* influences and hopefully to study "blocking" phenomena both *in vitro* and *in vivo*.

d) In collaboration with Dr. James Cohen (NCI-5561) we have studied using an allogeneic system differing at H-2 to evaluate the role of "blocking" sera on cell mediated immunity, hopefully to adapt this to syngeneic tumor systems having strong tumor antigens and *in vitro* assays, B10D2 mice immunized with Sa I (H-2^a; or B10A spleen cells yield peritoneal exudate cells (PEC) capable of lysing YAA-C1 H-2^a) Cr⁵¹ targets. These are highly specific, efficient and reproducible effects. A 40 - 100% decrease in cell mediated cytotoxicity achieved with hyperimmune anti B10A antisera - diluted out as far as 1:1000. This effect is shown to be specific since it did not block other H-2 reactions and the blocking activity was absorbed only with H-2^a cells.

e) Tumor antigens of Plasma Cell Tumors.

In collaboration with Drs. McCoy and Dean (Bionetics) we are searching for TSTA on BALB/c plasma cell tumors using a prototype the Ad; PC5 (A5) tumor of Potter. We have confirmed that A5 has strong TA

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of the transplantation type. Immunization to date has been achieved only by the aniline-mustard induced regression of R-5 tumors. Apparently these antigens are effective only when presented on living cells; they appear to be inactivated by sonication, by X-irradiation and by freeze-thawing. Cross-immunity has been achieved with LPC-1 and TEPC-15 but not with 3 other plasma cell tumors. We intend to study whether immunogenicity is related to the immunoglobulin selected by the R-5 tumor.

Honors:

Selected, Member Immunology Review Group, NCI 1972
Selected, Member Chemotherapy-Immune Council of Mt. Sinai
School of Medicine, New York City 1972

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PUBLICATIONS:

- Law, L., Appella, E., Strober, S., Wright, P., and Fischetti, T.: Induction of immunological tolerance to soluble mouse histocompatibility-antigens, Proc. Nat. Acad. Sci. (USA) 69: 1858-1862, 1972.
- Burstein, N. A., and Law, L. W.: Neonatal thymectomy and non-viral mammary tumors in mice. Nature 231: 450-452, 1971.
- Law, L. W.: Immune suppression and the induction of leukemia and solid tumors. Proc. Fifth International Symposium on Comparative Leukemia Research, 1973 (In press).
- Levy, H. B., Law, L. W., Rabson, A. S., Riley F., Margolis, S. and Asofsky, R.: The effect of polymosinic-polycytidylic acid on tumor growth. Third Internat. Symposium on Interferon, Lyon, France, 1972, pp. 289-298.
- Law, L. W., and Takemoto, K. K.: Specific transplantation antigens of murine neoplasms induced by visna and progressive pneumonia viruses. J. Nat. Cancer Inst. (In press, April 1973).
- Law, L. W., and Appella, E.: Immunogenic properties of solubilized tumor antigen from an RNA virus-transformed neoplasm. Nature (New Biology). (In press, April 1973).

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3200

Title: Factors influencing the induction and repression of primary tumors and the growth and inhibition of transplantable neoplasms. (Host and tumor cell variables).

Principal Investigator: Dr. Lloyd W. Law

Section: Office of the Chief

Lab/Branch: Laboratory of Cell Biology

Goal: 1) Studies of the solubilization and purification of histocompatibility antigens (H-2) and of virus-specific tumor antigens of the transplantation type (VSTA).

2) Studies of the biologic characteristics of VSTA and of the immune responses they engender.

Approach: 1) Through trial and error approaches, using digestion with papain and G150 Sephadex column fractionation we are attempting to establish a base line using genetic marked material (disociated spleen cells from several H-2 strains) in steps leading to purification of our solubilized transplantation antigens. In vitro inhibition by cytotoxicity, skin grafting, graft versus host and mixed Lymphocyte reactions are followed to judge the biologic character of our preparations. Through this knowledge we have gained, we have proceeded to solubilize and purify VSTA of several types, leukemia antigen, SV40 antigen and plasma cell antigen.

2) VSTA of different types (SV40, MSV, MLV and plasma cell types) have been identified and rejection responses in syngeneic mice, cross-reactivity, attempts at inhibition or facilitation of growth by T cells and B cells and antisera are being studied. Attempts are being made to develop meaningful in vitro assays such as Colony inhibition, ⁵¹Cr release cellular cytotoxicity, Winn assay for each tumor system that show specificity. Hopefully in this manner the meaning of in vitro assays will be related to in vivo reactions.

Progress: 1. H-2a antigens have been solubilized and purified that contain all of the known serologic specificities both public and private. These purified preparations are immunogenic and provide immunologic enhancement and tolerance. They appear to be deficient, though, in providing only humoral and not cell-mediated tolerance in which T-cell functions appear to be preserved. 2. Purified RBL-5 (leukemia) VSTA and SV-40 (fibrosarcoma) VSTA are found to be active in vitro assays and are specifically immunogenic in syngeneic hosts. 3. A microcytotoxicity assay for H-2 cellular cytotoxicity and a Cr⁵¹-release assays have been developed and perfected. These now allow meaningful studies of such immunologic phenomena as "blocking" and kinetics of the immune response.

Significance for Cancer Research (NCP Objective 3 Approach 4,5)

Level of Effort: Professional Man-Years: 3. (3 Research Associates have filed separate reports).

Serial No. NCI-5531
1. Macromolecular Biology
Section
2. Laboratory of Cell
Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Nature and aggregation of subunits of proteins.

Previous Serial Number: None

Principal Investigator: Samuel W. Luborsky

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1 1/2
Professional:	3/4
Other:	3/4

Project Description:

Objectives: As part of a study of the relationship between protein physicochemical properties and activity we wished to obtain a simple measure of protein stability. For this purpose we determined the resistance of the enzyme, L-asparaginase, to boiling.

Methods Employed: Analytical ultracentrifugation; enzyme assays using the nessler reaction; dialysis, fluorescence and absorption spectroscopy; polyacrylamide disc gel electrophoresis.

Major Findings: Studies were carried out on the effect of heat on the enzymatic activity and molecular structure of L-asparaginase. We observed that boiling of enzyme solutions produced inactivation which was slow enough to provide a simple and useful measure of enzyme stability. This inactivation is followed by dissociation of the enzyme, both processes by pseudo first order kinetics. Dissociation was monitored with sedimentation velocity experiments, by determining the percent of 7s "native" tetramer remaining in solution after a given time of boiling. Preliminary sedimentation equilibrium experiments indicated that the predominant species after dissociation had a molecular weight

of 24,500, similar to that of asparaginase monomer. Enzyme preparations bearing different lot numbers, obtained from the same commercial source, exhibited closely similar properties, including sedimentation behavior and specific catalytic activities. Unexpectedly they possessed different thermal stabilities, for unknown reasons. Nevertheless, among the five different lots of asparaginase studied, the ratios of their inactivation rates were closely similar following boiling, for high to low enzyme concentration (1.3), or for inactivation to dissociation (ca. 1.3). Thermal stability was less for enzyme dissolved originally in Tris buffer rather than in phosphate buffer, was unaffected by the addition of the substrate, asparagine, at saturating concentrations, or by dialysis prior to boiling. Stability was not related to such artifacts as adsorption to glass, since BSA precoating of containers prior to boiling had no effect on inactivation rates, nor was it related to the endotoxin contaminant present in these commercial preparations, since there was no effect upon addition of *E. coli* endotoxin prior to boiling to a preparation known to have low endotoxin contamination. Not unexpectedly, changes were observed in the fluorescence emission and difference spectra following boiling which are reminiscent of those observed upon denaturation by other means.

Significance to Biomedical Research and the Program of the Institute: The extent of aggregation of an enzyme is often the basis of a cellular control mechanism of activity. Studies of the stability of such interactions are thus important in attempting to understand such mechanisms and the nature of the forces responsible for the stability of the complex. Moreover, since L-asparaginase possesses significant anti-lymphoma activity, an understanding of its properties may help one to enhance this activity or to diminish some of its undesirable side effects.

Proposed Course of the Project: In order to obtain information on the nature of the subunit association sites and the mechanism of dissociation, the kinetics of inactivation and dissociation by boiling will be determined in the presence of additives which themselves may inactivate and/or dissociate the enzyme (e.g., urea and urea derivatives, monovalent and divalent metal ions, alcohols, detergents). The possible changes in substrate specificity due to these treatments, as well as the possible renaturation and reassociation of the enzyme, will be investigated. We must determine a more precise value for the molecular weight of the subunit produced by boiling, and determine its possible identity with asparaginase monomer produced by other means. Collaborative efforts will be initiated to test antilymphoma activity of any active fragments or derivatives of the enzyme which may be found in the course of this work.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 5531

Title: Nature and aggregation of subunits of proteins.

Principal Investigator: Dr. Samuel W. Luborsky

Section: Macromolecular Biology Section

Lab/Branch: Laboratory of Cell Biology, DCBD, NCI, NIH

Goal: To study the relationship between protein physicochemical properties and activity; to obtain a simple measure of protein stability.

Approach: For this purpose we determined the resistance of the enzyme, L-asparaginase, to boiling. Enzyme solutions were immersed in boiling water for designated times, then assayed using the nessler reaction. Boiling produced inactivation which was slow enough to provide a simple, useful, measure of enzyme stability. This inactivation was followed by dissociation. Dissociation was monitored with sedimentation velocity experiments, by determining the percent of 7s "native" tetramer remaining in solution after a given time of boiling. (Preliminary sedimentation equilibrium experiments indicated the predominant species after dissociation had molecular weight, 24,500, similar to asparaginase monomer.) Enzyme preparations bearing different lot numbers, obtained from the same commercial source, exhibited closely similar properties, including sedimentation behavior and specific catalytic activities. In some experiments the possibility of enzyme adsorption to glass was prevented by coating the containers with BSA; in others, E. coli endotoxin was added to preparations known to have low contamination with this material.

Progress: Unexpectedly, preparations bearing different lot numbers possessed different thermal stabilities, for unknown reasons. Nevertheless, among the five different lots of asparaginase studied ratios of pseudo first order decay rates were similar after boiling (high to low concentration - 1.3; inactivation to dissociation -ca. 1.3). Stability was less for enzyme dissolved originally in Tris rather than phosphate buffer; unaffected by addition of substrate, or by dialysis, prior to boiling. Lability was not an artifact of adsorption to glass, nor was it related to endotoxin contamination. Changes were observed in fluorescence emission and difference spectra following boiling which are reminiscent of those observed upon denaturation by other means.

Significance for Cancer Research (NCP Objective 6 Approach 1,2)

The extent of aggregation of an enzyme is often the basis of a cellular control mechanism. Studies of the stability of such interactions are important in attempting to understand such mechanisms and the nature of the forces responsible for the stability of the complex. Moreover, since L-asparaginase possesses anti-lymphoma activity, an understanding of its properties may help one to enhance this activity or to diminish some of its undesirable side effects.

Level of Effort: Professional Man-Years: 3/4

Serial No. NCI-5540
1. Macromolecular Biology
Section
2. Laboratory of Cell
Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The apparent specific volume of proteins.

Previous Serial Number: None

Principal Investigator: Samuel W. Luborsky

Other Investigators: Marc S. Lewis, NEI

Cooperating Units: None

Man Years:

Total:	5/8
Professional:	3/8
Other:	1/4

Project Description:

Objectives: As part of a study of the relationship between protein physicochemical properties and activity we wished to measure the apparent specific volume ($\bar{v}$) of a subunit-containing protein, asparaginase, under a variety of conditions, both before and after dissociation. These measurements will aid in the interpretation of the data obtained in the study of enzyme stability (Project Report NCI-5531), since knowledge of $\bar{v}$ is essential to the determination of molecular weights from sedimentation equilibrium data. The simplest current alternative method for determination of molecular weights, gel electrophoresis, is influenced by molecular conformation, the presence of covalently linked non-amino acid residues (e.g., carbohydrate), or bound ligands (e.g., cofactors, or detergents). Such results thus depend upon the assurance of purity of sample and its similarity to standards against which it is measured. This latter requirement is often particularly difficult to meet when one is confronted with the task of characterizing a new protein. Sedimentation equilibrium experiments are not influenced by these factors, and give accurate molecular weights as long as $\bar{v}$ is known for the sample under the conditions of interest. The $\bar{v}$ is calculated from precise measurements of solution densities.

Methods Employed: Measurement of protein solution densities, using a quartz oscillator densimeter; other methods, as listed in Project Report NCI-5531.

Major Findings: The densimeter was set up and connected to a large constant temperature bath which controlled temperature to better than $20.00 \pm 0.01^\circ\text{C}$ as read from a calibrated Beckman thermometer. Determinations of the densities of known solutions were too variable ($\pm 4/10^5$ in density). Better temperature control was deemed necessary and the bath and pumps were modified appropriately. A recording device to monitor temperature changes was designed (built by the Biomedical Engineering and Instrumentation Branch), which employs thermistors in both the constant temperature bath and in the densimeter. Preliminary results now appear promising, with variability much reduced (approaching $\pm 5/10^6$ in density).

Significance to Biomedical Research and the Program of the Institute: See Project Report NCI-5531.

Proposed Course of the Project: The $\bar{v}$ for asparaginase will be measured under all conditions required (cf. Project Report NCI-5531). With the experience gained in this project, measurements of $\bar{v}$ can be extended into other areas of current research interest where the nature of the proteins is less well defined and other substances are attached to the protein (e.g., immunoglobulins, glycoproteins, proteins solubilized from membranes, protein-detergent complexes). With these data it will be possible to obtain unambiguous molecular weights for such materials from sedimentation equilibrium measurements.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 5540

Title: The apparent specific volume of proteins.

Principal Investigator: Samuel W. Luborsky

Section: Macromolecular Biology Section

Lab/Branch: Laboratory of Cell Biology, NCI, DCBD

Goal: To study relationships between protein physicochemical properties and activity we wished first to measure specific volume ($\bar{v}$) of a subunit-containing protein, asparaginase, under variety of conditions, before and after dissociation.

APPROACH: Since knowledge of $\bar{v}$ is essential to the determination of molecular weights from sedimentation equilibrium data, these measurements will aid in the interpretation of the data obtained in the study of enzyme stability (Project Report NCI-5531). The simplest current alternative method for determination of molecular weights, gel electrophoresis, is influenced by molecular conformation, the presence of covalently linked non-amino acid residues, or bound ligands. Results thus depend upon the assurance of purity of sample and its similarity to standards against which it is measured. Sedimentation equilibrium experiments are not influenced by these factors, and give accurate molecular weights as long as $\bar{v}$ is known for the sample under the conditions of interest. The $\bar{v}$ is calculated from precise measurements of solution densities.

Progress: A quartz oscillator densimeter was set up and connected to a large constant temperature bath which controlled to better than $20.00 \pm 0.01^\circ\text{C}$ (read from calibrated Beckmann thermometer). Determinations of densities of known solutions were too variable ($\pm 4/10^5$ in density). Better temperature control was deemed necessary. Bath and pumps were modified appropriately. A recording device to monitor temperature changes was designed (built by the Biomedical Engineering and Instrumentation Branch), which employs thermistors in both constant temperature bath and in densimeter. Preliminary results now appear promising, with variability much reduced (approaching $\pm 5/10^6$ in density).

Significance for Cancer Research (NCP Objective 6 Approach 2,5)

The extent of aggregation of an enzyme is often the basis of a cellular control mechanism. Studies of the stability of such interactions are important in attempting to understand such mechanisms and the nature of the forces responsible for the stability of the complex. Moreover, since L-asparaginase possesses anti-lymphoma activity, an understanding of its properties may help one to enhance this activity or to diminish some of its undesirable side effects.

Level of Effort: Professional Man-Years:

3/8

Serial No. NCI-5554

1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

- Project Title: A. Characterization and measurement of soluble antigens associated with human neoplasms.
- B. Pathogenesis and pathophysiology of plasma cell neoplasms in mice and characterization of their immunoglobulin products.

Previous Serial Number: Same

Principal Investigator: K. Robert McIntire, M.D.

Other Investigators: Dr. Philippe P. Sizaret, I.A.R.C., Lyon, France
Dr. Charles Vogel, Uganda Cancer Institute, Kampala, Uganda
Dr. Thomas A. Waldmann (NCI-4002)
Dr. Richard Adamson (NCI-3509)
Dr. Charles Moertel, The Mayo Clinic, Rochester, Minnesota

Cooperating Units: I.A.R.C., Lyon, France
The Mayo Clinic, Rochester, Minnesota
Uganda Cancer Institute, Kampala, Uganda

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

A. Characterization and measurement of soluble antigens associated with human neoplasms. Objective 6 - Approach 1.

1. Studies of Alpha-Fetoprotein (α FP)

a. Quantitation of α FP in human disease: Using a sensitive double-antibody radioimmunoassay capable of detecting normal levels of serum α FP (<30 ng/ml) has helped to define those patients with hepatocellular carcinoma who were negative for α FP by double diffusion testing. We now know that 10-30% of hepatomas

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do not secrete α FP, but many of those previously thought to be negative are actually elevated but less than 1000 ng/ml which is the threshold of detectability for the double diffusion assay. The complete range of α FP can now be studied and this marker appears to be useful for monitoring disease at almost any point above the upper limit of normal. Evaluation of hepatoma in different geographic areas indicated that Chinese from Hong Kong had 91% with elevated α FP, Blacks from Uganda in East Africa had 85% and a group from the U.S. had 71%, but despite this difference the range of values was very similar.

In collaboration with Dr. T. Waldmann (NCI-4002) and Dr. Charles Moertel (Mayo Clinic) we have surveyed other neoplastic disease categories in an attempt to determine if α FP might be a useful marker. The following elevations were found: 10 out of 44 patients with pancreatic cancer, 15 of 80 gastric cancers, 9 of 197 colon cancers, 16 of 150 lung cancers, 34 of 48 embryonal cell cancers, 2 of 8 bile tract cancers, 0 of 14 esophagus or small bowel cancers, 0 of 150 breast cancers. These results would indicate that α FP may serve as a marker for selected neoplastic diseases.

b. α FP in primates with carcinogen-induced hepatoma: The complete sequence of hepatoma development has been followed in monkeys with weekly serum α FP determinations in collaboration with Dr. Richard Adamson. Elevation of α FP occurs several months before the tumor is clinically evident and this may serve as an ideal situation for testing the effect of chemotherapy and immunotherapy on early disease. The range of α FP values in monkeys is similar to that in humans and the level tends to plateau in the later stages of the disease.

c. Metabolism of α FP: We have continued the studies of α FP synthesis using tissues obtained at biopsy of the monkeys with hepatoma and also in tissue culture lines. Techniques for achieving α FP synthesis for up to 4 days in biopsy specimens have been worked out. The major problem is still one of cell enumeration. Catabolic rates in monkeys with hepatoma and elevations of α FP are faster than in normal monkeys so that the catabolism of α FP may be proportional to serum levels.

d. α FP in Ataxia-Telangiectasis (A-T): In collaboration with Dr. Thomas Waldmann we have shown that all of 20 patients with A-T had greater than normal values of α FP while 54 patients with other immunodeficiency diseases were all within normal limits. This constitutes evidence that the liver is not fully developed in

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these patients and supports the hypothesis that the primary abnormality in A-T is a defect in tissue differentiation that affects more than the thymus.

e. Immunochemical studies of α FP: α FP from seven different sources: hepatoma, fetus, teratocarcinoma, gastric carcinoma, colon carcinoma, pancreatic carcinoma and A-T have been shown to have antigenic identity by double diffusion in agar-gel.

2. Studies of other circulating markers: In collaboration with Dr. Charles Moertel and Dr. Wm. Go (Mayo Clinic) we have studied the simultaneous measurement of α FP and CEA. α FP was elevated in very few cases of colon cancer, but in 30% of a group with gastric cancer. CEA was elevated in 68% of colon cancer and 20% of gastric cancer. None of these patients had both markers although we have since found patients where both were elevated. Conclusions were that CEA and α FP together increased the detection of gastric but not colo-rectal cancer and cancer of the same organ can produce two different fetal antigens.

With the help of Drs. Griff Ross and J. Vaitakaitis (NICHD) we have set up a radio-immunoassay for HCG which has been shown to be a useful marker for selected neoplastic disease. We will correlate this test with results of α FP and CEA testing on the same serum samples. With Dr. Thomas Waldmann, we are setting up collaborative prospective clinical studies for the combined use of several markers to test the predictive value of each and in combination, especially for testicular and pancreatic cancer.

3. Studies of Lung Tumor Associated Antigens: Objective 5 - Approach 4

Further studies have been carried out with the antisera shown to detect antigens present in extracts of lung tumors, but not in normal lung. In collaboration with Dr. Philippe Sizaret (I.A.R.C.) additional lung extracts have been examined and a total of 59/74 have been positive for the antigen. Testing lacks the sensitivity for picking up the antigen in serum at this time. Attempts at purification of the antigen have localized it to one region on exclusion chromatography, but have not succeeded in concentrating the antigen. Immunization with more purified antigen has not yet succeeded in producing a more specific antiserum.

B. Mouse immunoglobulins.

1. Immunoglobulin (Ig) markers on plasma cell surface: Objective 3 - Approach 5.

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Studies in collaboration with Mr. Gerald Princler have examined the Ig determinants on a variety of plasma cell tumors. Plasma cells from 22 different transplantable mouse myelomas were tested by 14 different class-specific and type-specific anti-immunoglobulin antiserums for cytotoxicity effects using a trypan blue exclusion method. Seven IgF- κ tumors were all sensitive to anti-IgF and anti- κ antiserums, although there was variation in the degree of sensitivity. None of the other class-specific or anti- λ antiserums showed a cytotoxic effect against the IgF- κ cells. None of five IgG- κ tumors were sensitive to anti-IgG, but all were lysed by anti- κ serums and not by anti-IgH or the others. Two IgA- κ tumors were lysed by anti- κ but not by anti-IgA or the other antiserums. One IgM- λ tumor was lysed by anti-IgM anti- λ and anti- κ . None of the other class-specific or type-specific antiserums were effective. Three L chain-secreting tumors, one κ and two λ were tested and all 3 were killed by the anti- κ , but not by anti- λ .

Publications:

McIntire, K. R., and Vogel, C. L.: Quantitative studies of alpha-fetoprotein during progression of hepatocellular carcinoma. Second Conf. and Workshop on Embryonic and Fetal Antigens in Cancer, Oak Ridge, Tenn., 339-344, 1972.

Hull, E. W., and McIntire, K. R.: Serum alpha-fetoprotein in the United States. Second Conference and Workshop on Embryonic and Fetal Antigens in Cancer, Oak Ridge, Tenn., 317-321, 1972.

Mody, N. J., Vogel, C. L., Patel, I. R., and McIntire, K. R.: Rivanol precipitation technique for the detection of alpha-fetoprotein. J. of Lab. & Clin. Med. 80: 125-133, 1972.

Waldmann, T. A., and McIntire, K. R.: Elevated serum alpha-fetoprotein levels in patients with ataxia telangiectasia. Lancet 2: 1112-1115, 1972.

Braunstein, G. D., Bridson, W. E., Glass, A., Hull, E. W., and McIntire, K. R.: In vivo and in vitro production of human chorionic gonadotropin and alpha-fetoprotein by a virilizing hepatoblastoma. J. Clin. Endocrinol. Metab. 35: 857-862, 1972.

Esterhay, R. J., Shapiro, H. M., Sutherland, J. C., McIntire, K. R., and Wiernik, P. H.: Serum alpha-fetoprotein concentration and tumor growth dissociation in a patient with ovarian teratocarcinoma. Cancer 31: 835-839, 1973.

Serial No. NCI-5554

1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

Braunstein, G. D., McIntire, K. R., and Waldmann, T. A.: Discordance of human chorionic gonadotropin and alpha-fetoprotein in testicular teratocarcinoma. Cancer, accepted for 1973.

Robinson, E. A., Appella, E., and McIntire, K. R.: Structural studies of heavy chain from a mouse immunoglobulin M with antibody activity. J. Biol. Chem., accepted for 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5554

Title: Characterization and measurement of soluble antigens associated with human neoplasms

Principal Investigator: K. Robert McIntire, M.D.

Section: Immunochemistry

Lab/Branch: Laboratory of Cell Biology, DCBD

Goal: To develop immunochemical tests for detecting antigens associated with various human neoplasms so that they might be used for early detection and monitoring the therapy of cancer.

Approach: Using both known antigenic markers and others yet to be defined there is a need for quantitative tests that can describe the changes taking place in levels of the marker(s) that will correlate with changes in tumor size. We have developed two quantitative tests (accurate in different ranges) for the measurement of serum alpha-fetoprotein (α FP) and have been using these tests to screen large numbers of patients with various neoplastic and non-neoplastic diseases. Where elevations are found there is an attempt to follow serial serums for correlating with course of disease. The synthetic rate of α FP synthesizing tumors is being studied by in vitro incorporation of ^{14}C -labelled amino acids in material obtained by biopsy or resection. The catabolism of α FP is being studied in primates, both with and without carcinogen induced hepatoma. A radioimmunoassay for human chorionic gonadotropin (HCG) has recently been set up and will be used in a manner similar to the studies on α FP. Antiserums have been prepared against extracts of primary lung tumors as the first step in development of a quantitative test for antigens associated with lung cancer.

Progress: Screening neoplastic disease other than hepatoma and embryonal cell carcinoma has revealed elevated levels of α FP in significant numbers (>10%) of cancer of pancreas, stomach, biliary tract, and lung but not in colon, esophagus, small bowel, breast, lymphoma or leukemia. Use of combination tests for α FP and CEA will significantly increase the detection of gastric cancer, but not colonic. Measurement of α FP and HCG in teratocarcinoma indicated that treatment may selectively remove cells responsible for one marker more effectively than both, hence a real advantage in being able to monitor both α FP and HCG. Monkeys with induced hepatoma have rising α FP levels several months before the tumor is evident and successful treatment produces a fall in α FP. A lung tumor associated antigen has now been demonstrated in 59/74 extracts of primary lung tumors, but has not been demonstrated in the serum.

Significance for Cancer Research (NCP Objective 6 Approach 1)

The ability to estimate total tumor burden when the mass is less than the clinically detectable size is necessary for the rational use of chemotherapy and immunotherapy. This will depend on the use of biologic markers such as α FP, HCG, CEA and others which are yet to be discovered.

Level of Effort: Professional Man-Years: One

Serial No. NCI-5526

1. Macromolecular Biology
Section
2. Lab. of Cell Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cell surface changes in normal, DNA virus transformed and spontaneously transformed tumorigenic mouse cell lines.

Previous Serial Number: Same

Principal Investigator: Peter T. Mora

Other Investigators: Mrs. V. W. McFarland, Dr. Samuel Pancake, Dr. Alan Schultz, Guest Worker, Dr. Chris Fisher, Visiting Fellow, MBS, LCB; Dr. Robert Bassin, VLLB, CCP; Dr. John Bader, CH, DCCP, NCI; Dr. Peter Fishman and Dr. R. O. Brady, Jr., DMN, NINDS.

Cooperating Units: Department of Biochemistry, Oxford University, Glycoprotein Research Unit, University of Durham, England.

Man Years:

Total:	7
Professional:	5
Other:	2

Project Description:

Objectives: To elucidate certain biochemical changes on the cell surface which occur during transformation to malignancy in mouse cells.

Methods Employed: Tissue culturing methods (humidified CO₂ incubator, roller bottles, suspension cultures, etc.). Labeling of glycolipids and glycoproteins, also of nucleic acids and proteins, etc., with radioactive precursors during cell growth in culture. Cell homogenization and subcellular fractionation techniques such as isolation of cell membranes. Chemical isolation and characterization procedures, including thin-layer chromatography and gas-liquid chromatography. General enzymology techniques. Radioactivity counting procedures. Tumor take studies in mice.

Major Findings: I. Glycolipids and Glycoproteins. 1) Glycolipid biosynthetic enzymes. Previous findings (in collaboration with R. O. Brady's laboratory) indicated that in a number of cultivated mouse cells after transformation in culture by SV40 or polyoma viruses a decrease occurs in uridine diphosphate N-acetylgalactosamine:hematoside N-acetylgalactosaminyltransferase, an enzyme necessary for the stepwise elongation of the carbohydrate portion of gangliosides. There is no such decrease after "spontaneous" transformation in these cell lines after prolonged cultivation. Because of the known low efficiency of transformation by DNA viruses of mouse cells, the possibility exists that the above enzyme change is due to cell selection or to the tissue culture conditions employed. A thorough study was initiated, and the following major findings were obtained: a) Whole embryos, organs, tumors, etc., can be assayed for gangliosides and for the various sugar transferases, including the acetylgalactosamine-transferase, albeit with low accuracy. Mouse tissues contain generally a full complement of the various gangliosides and the various transferases, although the transferase activity is much lower than that of the cultured cells. b) Freshly started cultured cells can have great differences in the various transferase activity, depending on the method of initiation of culture, and on the number of passages in culture. Eventually, the established cell lines which survive are selected out, which then have fairly constant ganglioside content and high activities of the various glycolipid sugar transferases. These activities are then independent of minor permissible variations in tissue culturing conditions such as CO₂ content or pH, cell density at harvest, repeated passages at very high cell density, up to about 150 passages. c) Mouse cell lines transformed by SV40, generally but not always exhibit the decrease in the N-acetylgalactosaminyltransferase. Exceptions were found in addition to the normally growing "phenotypic revertants", such as "cryptic transformants" which have SV40 DNA detectable only by DNA:DNA hybridization but no other SV40 gene expression, or a cell line which was highly selected out by resistance to BUDR and repeated cloning. Also, a mutant cell line, which was SV40 transformed but temperature sensitive in some of its growth properties in culture, showed no decreased N-acetylgalactosaminettransferase at the permissive temperature. These cell lines were graciously contributed by various investigators from NIH, NYU, Cold Spring Harbor, Berkley, Baylor University, the Wistar Institute, etc. d) In our laboratory we undertook a systematic investigation to compare spontaneously or virally transformed AL/N mouse cell lines to correlate tumorigenicity with cell growth in culture, and with certain cell surface properties. Cells were subjected to two types of "selection pressures", one when passing the cells through the syngeneic host and the other when reestablishing cultures from the tumors. The spontaneously transformed cells had very high tumorigenicity, which increased even more after repeated passages through the

syngeneic host. The SV40 transformed cells had low tumorigenicity in the immunocompetent host, while the tumorigenicity was greater in X-irradiated host. Tumorigenicity of the virally transformed cells increased considerably after repeated passages through the syngeneic host. This property was correlated then with the influence of cells on each other's growth in culture, and with the activity of a key cell surface glycolipid transferase. In the SV40 transformed cells the expression of T antigen and of susceptibility to lysis by specific antisera was also followed. The conclusions were reached that the glycolipid transferases are probably under control of chance in the two selection conditions, while the SV40 genome and some of its expression as generally tested by virologists (T antigen, susceptibility to serum mediated cytotoxicity - see below) is maintained. Tissue culture growth properties such as the rate of cell growth, "saturation density", however, is not significantly different between the spontaneously transformed and virally transformed cell lines and in their derivatives after multiple in vivo passage. Thus, in many cases it can be completely erroneous to refer to cells which grow to higher saturation density in culture as malignant cells, and the expression of other cell surface properties must be dominating in tumorigenicity and in rejection (cf. see below the remarks on viral induced tumor specific antigens). Also we could not find yet any consistent change in the size and amount of cell glycoproteins, in sialic acid content of cells, and in some of the enzymes participating in glycoprotein biosynthesis. The work on glycoproteins and in the pertinent enzymes, however, is still in a preliminary stage and work is being intensified in these directions. The glycolipid (ganglioside) and glycolipid transferase changes we reported before on virally transformed cells are superimposed changes on differences due to chance effect of selection dependent on the procedures employed in establishing cell lines. e) RNA tumor viruses can transform cells virtually completely within one or two cell generation, and the above mentioned problems of selection and chance can be mainly avoided. Therefore, Swiss 3T3 cells were infected with Moloney sarcoma virus at a high multiplicity of infection and levels of four glycosyltransferases involved in ganglioside biosynthesis were subsequently determined. By day 2 over 95% of the cells were transformed; thereafter, there was a steady decrease in N-acetylgalactosaminyltransferase activity. At day 4 there was a 50% reduction compared to uninfected cells and at day 7, an 80% reduction. There was no decrease in the other glycosyltransferases, and infection with Moloney leukemia virus had no effect. Thus transformation of Swiss 3T3 cells by this RNA tumor virus produces a similar alteration in ganglioside biosynthesis as is generally seen with DNA tumor viruses. The implications of this specific change, reduced N-acetylgalactosaminyltransferase activity, which occurs subsequent to viral transformation are being investigated in relation to growth and behavior of these cells both in culture and in vivo.

The decrease in galNAc transferase occurs after the morphological transformation induced by the MSV becomes evident. Therefore, it is apparent that the change in growth property of MSV transformed cells is not a consequence of the decrease in this enzyme level; but it is possible that the decrease in enzyme level is one delayed phenotypic expression of an MSV gene functioning in the transformed cells. Since infection with MLV alone is not associated with a decrease in the enzyme activity at a time when virus is being produced, replication of the entire leukemia virus genome is not sufficient to cause the specific change in N-acetyl-galactosaminyl transferase activity. Prior cell transformation by MSV, evidence for functioning of that portion of the sarcoma virus genome which induces transformation in cell morphology, however, appears to be necessary for this change in enzyme activity.

It is of interest that the same enzyme alteration, a decrease in galNAc transferase activity, is observed following an RNA virus induced transformation as is seen in DNA virus induced cell transformations of established mouse cell lines. This is especially noteworthy since, as discussed above, the change observed in enzyme activity could well serve as one of the biochemical markers of sarcoma virus infection, or generally of viral functions which are responsible for the transformation of cells in culture. For the (se) function(s) a portion of the sarcoma virus genome which is present in the defective sarcoma may be sufficient, thus the biochemical marker may distinguish this genome or its portion from the genomes of transformation defective leukosis type viruses.

It should be emphasized that these data pertain to a single system of RNA virus-induced transformation of an established mouse cell line which was selected on the basis of suitability for study. Recently, cloned Balb/c 3T3 cells were also quantitatively transformed with MSV. As before, galNAc transferase activity decreased following morphological transformation. A similar decrease in this enzyme activity was observed in an S+L- cloned Balb/c cell line stably transformed in the absence of leukemia virus and cultivated for about a six month period. Thus the change in the activity of galNAc transferase occurs upon MSV transformation in at least two different mouse cell lines, and the decrease in the galNAc transferase activity is a very stable heritable phenotypic property of single cell clonal progenies.

Although the mechanism of this enzyme alteration is unknown, when homogenates of uninfected and infected fully transformed cells were mixed together in equal proportions there was no significant deviation from the expected galNAc transferase activity, indicating no excess cytoplasmic enzyme inhibitor in the transformed cells or activator in the untransformed cells.

Work is being continued to clarify the biochemical mechanism, and to compare recently available temperature sensitive RNA virus transformed cell lines. On these latter cell lines, we are also investigating glycoproteins, and certain glycoprotein sugar transferases (work in collaboration with Drs. Bassin and Bader).

II. Effects of Halogenated Carbohydrates as Modifiers of Glycoprotein Biosynthesis (project in collaboration with Dr. Paul W. Kent, University of Durham, England). The objective of this project is to find a novel approach to alter carbohydrate metabolism leading to glycoprotein and glycolipid biosynthesis in cultivated mouse cells, when including in the growth medium halogenated N-acetylglucosamine.

Previous work showed that N-fluoracetylglucosamine for one day at 0.5 mM concentration reduced the specific activity of ^{14}C -glucosamine in whole cells grown in tissue culture. Both spontaneously transformed and virally transformed cells were affected such equally. We now found (with Dr. A. Schultz) that glucosamine rate of entry is not reduced: T AL/N cells in log phase of growth were exposed up to 1 hour to 1 $\mu\text{Ci/ml}$ of ^{14}C -glucosamine in the presence or absence of 0.5 mM N-fluoracetylglucosamine. The rate of uptake of glucosamine was the same: 160 picomoles/hr/mg protein.

By differential label uptake in simultaneous labelling with [C^3H_3] thymidine or [^3H] uridine with [1 - ^{14}C] glucosamine, we found that the fluoro-N-acetylglucosamine had no significant effect on DNA or RNA synthesis. Similar experiments with[G - ^3H]-L-threonine also showed no effect on amino acid uptake.

When after six day treatment with the same concentration (0.5mM) fluoro-N-acetylglucosamine the incorporation of ^{14}C -glucosamine into isoalted glycoproteins was measured, unexpectedly a large (5 fold) increase of specific activity was observed in both spontaneously and virally transformed cells.

We are investigating the mechanism of such changes. We do not know yet how to account for these alterations in glycoprotein biosynthesis by the apparently non toxic doses of the fluorinated aminosugar. Our hope is that understanding better the biochemical mechanisms, we may develop approaches to influence carbohydrate metabolism of apparently normally growing cells, and alter the resulting glycoproteins and glycolipids, and hence some of the surface characteristics of cells, including some biological properties, such as immunogenicity.

III. Surface Antigens Associated with SV40 Transformed AL/N Mouse Cells (with Dr. Samuel J. Pancake). The plan of research a year ago was to isolate and chemically characterize the cell surface antigens which appear following SV40 transformation of

tissue cultured AL/N mouse cells. Previous work by Smith and Mora indicated that such antigen activities were not only readily detectable, but that by means of a serum mediated cytotoxic assay they could be quantitatively determined. In addition it appeared that of several SV40 transformed cell lines tested all reacted positively in the assay, regardless of strain or species origin. Furthermore, studies on SV40 transformed murine tissue culture cells suggested that antigen materials were readily recovered in soluble form and in good yield following treatment of the intact cells with papain.

Several months were spent in adapting tissue culture techniques and the cytotoxic assay to be more quantitative and reproducible. Various small modifications of the assay were made in order to try to obtain more reproducible results. It was necessary to produce new antisera against SV40 transformed cells. AL/N mice were immunized with long term tissue cultured SV40 transformed AL/N cells. Antisera activities were determined by the cytotoxic method. Sera from animals immunized six or more times with 1×10^6 cells at weekly intervals reacted almost as strongly with certain non-transformed cell lines (but not with T AL/N) as with SV AL/N. There was some suggestion that among the sera obtained from several groups of animals that those immunized with trypsinized cells possibly showed more cross reaction than those immunized with cells directly scraped off of the plates. Also possibly the sera with lower titers showed better specificity. Subsequently animals were immunized with scraped cells and the number of injections was decreased. The sera obtained usually had a cytotoxic titer against SV AL/N which was several fold higher than that observed for other cell lines. An exception was Py AL/N which usually showed a strong cross reaction.

Using the more specific sera, studies directed at preparing solubilized antigen were carried out. Cells broken open by nitrogen bombing and immediately assayed retained no measurable capacity to bind antiserum. Limited digestion of intact cells with papain under a variety of conditions produced no detectable solubilization of antigen, although the recovered cells appeared to have a greatly decreased antigen content. Similar experiments after treatment with trypsin gave similar results.

Further studies of antiserum specificity indicated that the sera had only weak cytotoxic effects against SV40 transformed hamster or human lines. Absorption studies are in progress to try to determine whether these lines lack the antigen(s) the anti sera are directed against, or whether antibody is bound but is unable to effect lysis of SV40 transformed cells of non murine origin.

Serum mediated cytotoxic assays were carried out on the previously mentioned SVS AL/N cell lines which were passed through

repeatedly as tumors in the syngeneic animal. No change in the killability of the derivative cells implicate cell mediated factors in selecting for the above discussed increase in tumorigenicity in the cells which survive the in vivo passage.

The results of our work this year on the surface antigens detectable by serum mediated cytotoxicity admittedly represent a certain retrenchment from some of the published findings of Smith and Mora. The reason for this is that with Dr. Pancake the emphasis was on critical and careful reexamination of the earlier findings, especially to what extent the various antisera produced are truly specific when assayed in more quantitative manner. Absorption and inhibition techniques will be similarly reexamined in a more careful and critical manner before conclusion will be reached on the appropriateness of these serum mediated cytotoxic assays for biochemical examination to be used for attempted purification of tumor transplant antigens in this particular animal model system.

Significance to Biomedical Research and the Program of the Institute: Studies of cell surface biochemical changes which relate to loss of contact inhibition in culture and to acquisition of tumorigenic potential in vivo are of interest, especially when they may lead to understanding of changes in molecular mechanism (enzymology) which appears to be reducible to heritable changes on control processes on the DNA level in the virally induced transformation of cells.

Proposed Course of the Project: There will be an attempt to further clarify the relevance of these biochemical observations to spontaneous and to viral induced transformation of cells and to correlate various enzyme activity changes with other cell membrane biochemical changes (glycoproteins, virus induced antigens as detectable by immune cytotoxic technique, etc.). We also plan to relate these changes, and the changes caused by the amino-sugar biosynthesis modifiers, to changes in cell immunogenicity and in tumorigenicity in syngeneic and allogeneic mouse systems.

Honors and Awards:

P. T. Mora was invited to the First McInnes International Symposium on Generation of Subcellular Structures; and to the First Oxford International Symposium on Membrane Mediated Information.

Publications:

Smith, R. W., and Mora, P. T.: Cytolytic microassays and studies of SV40 tumor-specific transplantation antigen. Virology 50: 233-246, 1972.

Fishman, P. H., Brady, R. O., and Mora, P. T.: Altered glycolipid metabolism related to viral transformation of established mouse cell lines. In Cancer Lipids, Biochemistry and Metabolism. Randall Wood, Editor, American Oil Chemist Society, 1973. In press.

Brady, R. O., Fishman, P. H., and Mora, P. T.: Membrane components and enzymes in virally transformed cells. Fed. Proceedings 32: 102-108, 1973.

Mora, P. T.: Cell growth regulation. In Membrane Mediated Information; Biochemical Functions. P. W. Kent, Editor, Medical Technical Publication, Oxford & Lancaster, England. In press.

Kent, P. W., and Mora, P. T.: Biosynthetically-induced modification of the structure of membrane-bound oligosaccharides. In Membrane Mediated Information: Biochemical Functions. P. W. Kent, Editor, Medical Technical Publications, Oxford & Lancaster, England. In press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 5526

Title: Cell surface changes in normal, DNA virus transformed and spontaneously transformed tumorigenic mouse cell lines.

Principal Investigator: Dr. Peter T. Mora

Section: Macromolecular Biology Section

Lab/Branch: Laboratory of Cell Biology, DCBD, NCI

Goal: To elucidate certain biochemical changes on the cell surface which occur during transformation to malignancy in mouse cells.

Approach: Tissue culture cultivated mouse cell lines, before and after spontaneous or virally induced transformation in culture, were compared in their cell surface biochemical properties and in their growth properties in vivo (tumorigenicity) and in culture. Special attention was directed to the outermost cell membrane components containing the glycoproteins and glycolipids and to the biosynthetic pathways.

Progress: Serial passage through syngeneic mice was found to alter tumorigenicity in both the spontaneously and the virally transformed cells, in a manner which can be rationally expected from selective survival and outgrowth of the cells depending on the conditions used. Metabolic modifiers were found for carbohydrate incorporation into cell glycoproteins. The modifiers are non-toxic to the cells, but depending on the method of treatment can selectively alter (either increase or decrease) cell membrane glycoprotein biosynthesis and thus certain cell surface properties.

Significance for Cancer Research (NCP Objective 3 Approach 2,5)

Cell surface biochemical changes, especially in glycoproteins and glycolipids can help to delineate the nature of oncogenic cell transformation in carcinogenesis, could lead to cellular and organismal modifiers of the transformation and promotion process, and to elucidation of immunologic aspects of transformation.

Level of Effort: Professional Man-Years: 5

1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Genetic and biochemical mechanisms controlling protein synthesis in mouse plasma cell tumors and other well-differentiated neoplastic cells

Principal Investigator: J. Frederic Mushinski, M.D.

Previous Serial Number: Same

Other Investigators: Dr. M. Potter (NCI-5556)
Dr. Carl T. Hansen (DRS-VRB-5)
Dr. P. O. Periman, George Washington University
Dr. Matthew D. Scharff, Albert Einstein School of
Medicine

Cooperating Units: George Washington University
Albert Einstein School of Medicine

Man Years: Professional - 1
Other - 1

Project Description:

Objectives:

A. To study transcriptional and translational control mechanisms operating in mammalian cells using rodent tumors in vivo and in vitro as models of human cancer and normal tissues.

B. To develop new animal models of immunoglobulin synthesis and secretion in inbred rats.

C. To study the feasibility of immunotherapy of growing plasma cell tumors.

Methods Employed:

A. Control Mechanisms:

1. IgA half molecule mouse myeloma proteins

Three approaches are being used to understand the genetic and biochemical events responsible for the synthesis in a few mouse plasmacytomas

1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

of IgA molecules which are excreted into the urine and consist of a single heavy chain disulfide bonded to a single light chain. Two tumors have been selected as prototypes of a typical, polymeric IgA producer (Adj PC-6A) and a IgA half molecule producer (Adj PC-6C). On the protein structural level we are preparing quantities of each protein for detailed sequence studies. On the cell culture level we are going to study whether mutagenic agents can cause half molecule producing clones to arise from polymeric IgA producers and vice versa. On the subcellular level we are preparing tRNA from these tumors for comparison on reverse phase chromatography and for study as mammalian suppressor RNAs.

2. Transfer RNA from mouse myeloma and rat hepatomas

The high resolving power of reversed phase chromatography of tRNAs is also being used to look for a biological function correlated with the variations in tRNA chromatographic pattern we have seen among different myelomas and hepatomas. We are beginning to compare the tRNAs from two light chain producing myelomas that make light chains with only two amino acid differences in the entire 214 amino acid residues. Another tRNA comparison is underway between two tumor lines developed from a single clone of IgG producing plasmacytoma cells, one secreting its characteristic protein and the other having mutated spontaneously to a line of cells no longer secreting any myeloma proteins. tRNA profiles from 3 Morris hypatoma lines are being studied in comparison with normal and regenerating rat liver.

B. Induction of Rat Tumors:

1. In an attempt to produce a series of immunoglobulin secreting tumors in rats analogous to those induced by intraperitoneal mineral oil in BALB/c mice, we have been injecting Pristane intraperitoneally in rats of 17 different inbred strains. In hopes of increasing the effectiveness of this procedure one group of rats of all strains has also been treated with burro anti-rat thymocyte serum.

2. Another attempt to induce rat tumors utilized subcutaneous and intraperitoneal injections of lead acetate in inbred Sprague-Dawley rats.

C. Immunotherapy: Xenogeneic antisera to mouse and human light chains have been selected for cytotoxicity in vitro using Trypan Blue exclusion as index of viability.

Major Findings:

A. Control Mechanisms:

1. Antisera have been successfully prepared in reagent amounts that can distinguish between polymer-type and half molecule-type IgA molecules.

These proved to be most difficult to prepare in more than analytical quantities and can be made only in A strain mice. The small amounts of antiserum available from mice necessitate adapting the Scharff techniques of cloning and secreting product identification in soft agar to a more micro scale, which is still undergoing development.

2. A newly developed, more rapid reverse phase chromatography method (RPC-5) has been adopted for faster tRNA comparisons. This has necessitated repeating many of the comparisons done earlier with the rat hepatomas (using RPC-2). This new method has elucidated the presence of four Phe-tRNA peaks in rat hepatomas instead of the two peaks described previously. Furthermore, one minor Phe-tRNA peak has been described as "tumor specific" by several laboratories since they could not demonstrate its presence in normal rat liver. Using a crude enzyme preparation which exhibits more amino-acylating activity than the more purified preparation usually used by us and by others, we have found this peak of Phe-tRNA in normal liver as well as hepatomas. It is not yet clear whether this is due to an additional aminoacyl synthetase for phenylalanine or the presence of some tRNA modifying enzyme either of which might be eliminated by the purification step. No differences among Phe-tRNAs of mouse tumors has been observed.

B. Rat Tumors:

1. While the large scale experiment giving Pristane to 400 rats is still in its infancy a pilot experiment using 25 rats of 4 strains has produced only one transplantable tumor. This developed in a Wistar rat and is not completely characterized as yet. It seems probable that it is a reticulum cell sarcoma which does not secrete immunoglobulin.

2. Rather unexpectedly we found large, rapid growing fibrosarcomas developed in 3 of 7 male and 0 of 5 female inbred Sprague-Dawley rats at the site of subcutaneous injections of 4% lead acetate. These tumors developed between 9 and 12 months after initial injection, but were preceded by an acute, granulomatous, often ulcerating reaction to the lead salt which developed a week after injection and subsided after four weeks. The tumors grew to about 1/3 total body weight of the rat before death ensues. Local growth rather than metastasis in invasion seems to be the rule which permits surgical removal of the tumor. Transplantation by subcutaneous trocar implantation of minced tumor is easy into syngeneic animals of both sexes but not into several other inbred rat strains. Interesting to note is that all of the remaining 4 males and one of the 5 females now have very small, apparently very slow growing tumors at injection sites which may be akin to a carcinoma-in-situ state.

Significance to Biomedical Research and the Program of the National Cancer Institute:

A. Our studies of tRNA and other mechanisms controlling protein synthesis

1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

may confirm our suspicion that certain of these mechanisms may be diverted by neoplasia to abnormal functions and through them we may find a handle to treat neoplastic cells by specially tailored chemotherapy. So far, however, our Phe-tRNA data make neoplastic-normal tRNA differences appear less significant than formerly thought. The studies, nonetheless, have increased our understanding of protein synthesis in mammalian cells, and especially in conjunction with newly available mammalian mRNA, continued studies of tumor tRNA are bound to yield further such insights.

B. Our survey of inbred rat strains is beginning to afford baseline hematologic values and will soon make comparative immunoglobulin characteristics for all these strains available to the scientific community. If we are successful in producing myelomas in rats which seem to be less redolent with endogenous and iatrogenic viruses, we will be able to compare the pathogenesis with that in BALB/c mice to further our ideas of the mechanism of mineral oil induction of plasmacytomas.

The subcutaneous fibrosarcomas induced by lead salts are not previously described and offer many possibly fruitful avenues for further exploration: carcinogenicity of other environmental lead compounds, genetic and sexual differences in susceptibility to lead acetate sarcoma induction (Wistar rats similarly treated are reported to get kidney capsular sarcomas but no injection site tumors), tumor antigen comparison among a series of lead induced tumors, and several possibilities can be tried to induce the slow-growing subcutaneous tumors to become more malignant: more lead acetate, antithymocyte serum, and "promoter serum" from animals bearing large growing tumors of the same type.

Proposed Course

A. Continued pursuit of the genetic and biochemical origins of the half molecule IgA myeloma protein is proposed along the three courses outlined above: protein structural comparisons, mutation studies in cell culture, and tRNA comparisons will be pushed in the direction that the results will indicate or phased out if unrevealing.

B. The protocol study outlined for the large scale trial of mineral oil as an inducer of plasma cell tumors in rats will be finished during this next fiscal year. If it continues to be unfruitful in producing immunoglobulin producing tumors, it will be terminated. Another approach to these tumors will be attempted concurrently using Abelson virus, as has been successful in BALB/c mice.

The lead acetate tumors will be explored along the lines indicated above for usefulness as a series of tumors induced by inorganic carcinogens as opposed to polycyclic hydrocarbons or viruses.

C. Immunotherapy with anti-light chain antisera has been abandoned.

Serial No. NCI-3222

1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

An exploration of the usefulness of RNA extracts in inducing tumor immunity against plasma cell tumors in mice well controlled for H2 types and immunoglobulin allotypes is being considered.

Relationship to National Cancer Plan:

Objective - 3

Approach - 1

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3222

Title: Genetic and biochemical mechanisms controlling protein synthesis in mouse plasma cell tumors and other well-differentiated neoplastic cells.

Principal Investigator: J. Frederic Mushinski, M.D.

Section: Immunochemistry

Lab/Branch: Laboratory of Cell Biology, DCBD

Goal: To study transcriptional and translational control of protein synthesis in mammalian cells using well-differentiated rodent tumors as models of human cancer and normal tissues.

Approach: We have selected for study several established lines of mouse plasma cell tumors which secrete immunoglobulins with particular differences and similarities. The biochemical components which control the synthesis of these proteins are then dissected from these tumor cells and compared. The first component studied, transfer RNA (tRNA), showed significant differences when the Ser-tRNAs and Leu-tRNAs from light chain producers and from IgA producing tumors were compared using reverse phase chromatography. Similar chromatographic comparisons from rat hepatomas also showed significant differences. The present approach is to compare tRNAs from the following: 1) two tumors secreting light chains with only two amino acid differences; 2) two tumors which secrete IgA molecules with identical light chains but different heavy chain "constant regions;" and 3) cloned tumor lines before and after a mutation causing protein secretion to cease. We hope to see if we can establish a correlation between tRNA profiles and some of the observed differences in protein production and to see if these tRNA populations can significantly alter the translation of purified immunoglobulin messenger RNA in a cell-free system. Attempts to induce plasma cell tumors in inbred rats for similar studies is also being undertaken.

Progress: We have found evidence for a second peak of Phe-tRNA in normal rat liver, which has been reported to be found only in tumors and never in normal or regenerating liver. Probably the preparation of the enzyme used to charge phenylalanine to the tRNA is responsible for the discrepancy in these observations, and we are presently looking for an additional Phe-tRNA synthetase not previously described of an enzyme responsible for altering a portion of the one normal Phe-tRNA.

Induction of rat tumors has not produced any plasmacytomas to date, but a transplantable leukemia or reticulum cell sarcoma in Wistar rats has been found after injecting intraperitoneal mineral oil, and subcutaneous injections of lead acetate have produced fibrosarcomata which kill the host and are transplantable only into syngeneic rats.

Significance for Cancer Research (NCP Objective 3 Approach 1).

Further understanding of how mutations affect or are affected by certain elements in the intracellular machinery of protein synthesis will show us those aspects which may be uniquely important to neoplastic cells and, therefore, may be prevented or inhibited and lead to additional tools for cancer prevention or treatment.

Level of Effort: Professional Man-Years: One

Serial No. NCI-3202

1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Pathogenesis of plasma cell neoplasia in mice; immunochemical characterization of antigen-binding myeloma proteins; genetic basis of immunoglobulin diversity.

Previous Serial Number: Same

Principal Investigator: Michael Potter, M.D.

Other Investigators: Dr. R. Asofsky (I:LGAR-116c)
Dr. R. Lieberman (I:LCI-15c)
Dr. S. Rudikoff (NCI-5553)
Dr. David R. Davies (A:LMB-7)
Dr. C.P.J. Glaudemans (A:LC-7)
Dr. Don W. Bailey (Roscoe B. Jackson Lab.,
Bar Harbor, Maine)
Dr. Marshall Sklar (I:LVD)

Cooperating Units: None

Man Years:	Total:	4
	Professional:	1
	Other:	3

Project Description:

I. Pathogenesis of Plasma Cell Tumor Development.

Plasma cell tumors can be induced in 60 to 70% of BALB/c mice by the intraperitoneal injection of various mineral oils or pure hydrocarbons (e.g. pristane). With this model system 3 important general factors appear to participate in plasmacytoma development: 1) Genetic susceptibility, revealed by the strain susceptibility of inbred strains BALB/c and NZB and the resistance in most other strains (C57BL, DBA/2, C3H, ALN); 2) An abnormal tissue micro-environment - the mineral oil granuloma which promotes plasmacytoma development; 3) An initiating factor which could be due to replication of an intrinsic latent virus or a spreading somatic infection by an endogenous virus; 4) Antigenic stimulation.

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2. Immunochemistry Section
3. Bethesda, Md.

A. Genetic factors in mineral oil induced plasmacytoma formation.

1. Search for susceptibility genes.

Strain C57BL/6 mice are resistant to plasmacytoma formation by mineral oil. The availability of the 7 Bailey Recombinant strains derived from a cross between C57BL/6 and BALB/c provides a potential means to identify genes involved in susceptibility. The 7 Bailey strains have been tested initially for susceptibility to BAYOL-F and the CXBG and CXBJ strains developed a high incidence of tumors. The CXBD strain was inconclusive, the others were essentially negative. The study was complicated by a high incidence of non specific deaths, and is now being repeated using pristane which induces tumors in a shorter time. The CXBG and CXBJ mice have the same IgC_H genes and linkage group VIII, and thus differ from the other strains. On this basis, an F₂ population of BALB/c X C57BL/6 is being raised and F₂ mice homozygous for IGC_H (BALB/c) and bb will be tester for susceptibility, as controls_H mice bearing the other alleles will be used.

The recent observation in our laboratory that Abelson virus (C-type RNA virus complex that contains Moloney Virus, and LDH) induces 15 to 20% plasmacytomas rapidly provides an additional means to assess the Bailey RI strains. This method also will obviate factors that interfere with establishing a true incidence. Accordingly Bailey RI strains will be given 1 injection of Pristane and MLV-A 5 weeks later.

2. Possible role of somatic mutation.

The compound ethyl methane sulfonate which is a potent mutagen in mice has been tested for its co-carcinogenic effect in plasmacytoma formation. In three experiments it has been found that EMS 1) decreases the latent period of plasmacytoma development 2) slightly or moderately increases the yield of plasmacytomas. A final experiment in this series is being completed, in which EMS is being tested for its ability to decrease the latent period in mice given only 2 injections of the relatively weak light mineral oil.

B. Abnormal tissue microenvironment.

In the last year we have completed a series of studies on the transplantability of primary plasmacytomas into normal mice and mice that have previously received one or two intraperitoneal injections of mineral oil. Of the first 15 tumors tested all have

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grown rapidly in the mineral oil conditioned mice and only 3 have grown in normal mice. Cell doses ranging from 10^5 to 10^2 cells were given and 10^2 and 10^3 cells have been effective in transplanting the tumors. These results dramatically indicated that the conditioned peritoneum either provided special factors that favored plasma cell tumor growth or that because of some alteration in physiology, permitted plasma cell tumor growth e.g. by providing a protected environment.

1. Growth factor. Recent studies by Namba and Hanaoka have indicated the intraperitoneal injection of Freund's adjuvant results in the formation of a growth factor that stimulates plasmacytomas in vitro. We have observed that certain plasmacytomas grow more rapidly in primed mice than in controls. Priming locally increases the transplantation efficiency.

2. Local Abrogation of Established Immunity. We have completed a study on the effect of priming on established immunity to the AS, PCS plasmacytoma and have found that primed mice challenged intraperitoneally with plasmacytomas are essentially unable to eliminate tumor cells even though immune processes slow tumor growth. Primed mice challenged subcutaneously are resistant to challenge.

To relate this abrogation effect to the primary plasmacytoma the general question of cross-reacting vs individual antigens on plasmacytomas is being studied. Six mineral oil induced plasmacytomas are being tested for tumor specific transplantation antigens, and cross challenges of immunized mice are being carried out. Preliminary evidence indicates some tumors can cross protect. In addition, plasmacytomas induced by the MLV-A are being introduced into the system to assess the possible role of viral antigens. Pursuit of these studies should lead to the identification of the nature of antigens on the plasmacytomas and these facts may then be helpful in assessing the potential role of local immune suppression on plasmacytoma development.

C. Search for an Initiating Factor.

In collaborative studies with Drs. Sklar and Rowe we have found Abelson virus (a C-type RNA-virus) infection established 5 to 8 weeks after a single injection of pristane induces plasmacytoma formation in 3 to 7 weeks. The incidence of plasmacytomas is 15 to 20%, and is associated with the concurrent development of lymphosarcomas (B-cell immunoglobulin producing lymphomas). Eleven Abelson plasmacytomas have been characterized for myeloma protein production, 9 produce IgA class proteins 2 IgH ($\gamma 2b$)

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proteins. The identity of the first virus that plays a role in plasmacytoma formation opens a number of experimental possibilities 1) rapid means to find genetic basis of susceptibility 2) possible means for inducing tumors making specific types of antigen-binding myeloma proteins and 3) determining if an endogenous BALB/c virus is the true initiator of plasmacytomas.

Studies in progress are to determine 1) if tumor plasmacytomas can be induced in resistant strains e.g. C57BL/6, (BALB/c x DBA/2)F₁ and the 7 Bailey RI strains; 2) role of viral dose; 3) relationship of virus infection to the duration of pristane priming; 4) role of preimmunization on induction of tumors; 5) search for an antigenic relationship of between Abelson virus plasmacytomas and mineral oil only plasmacytomas; 6) attempts to rescue an endogenous virus from mineral oil induced plasmacytomas by Moloney LV and to determine the role of LDH-virus (a known component of Abelson virus).

D. Antigenic Stimulation.

Germfree BALB/c mice treated with mineral oil develop plasma cell tumors only rarely and then usually at an advanced age. In collaboration with Dr. Richard Asofsky (I:LGAR-116c), we have found that BALB/c mice monocontaminated with Salmonella tel aviv survive moderately well under germfree conditions. The Salmonella tel aviv strain was chosen because it had been previously observed that 2 myeloma proteins specifically precipitated with the O-specific antigen produced by this species of Salmonella. Despite the fact that a number of the mice monocontaminated with Salmonella tel aviv developed unusual illnesses months after the initiation of the monocontamination which unfortunately resulted in the loss of many of these animals before autopsies could be done, we nonetheless were able to observe a slightly increased incidence of plasma cell tumors. During the course of these experiments, however, it became apparent from other publications and our experiences with monocontamination that monocontamination such as we had initially performed was not the best means for studying antigenic stimulation in germfree BALB/c mice. It was considered that antigen feeding would be more effective. Heremans and colleagues in Belgium have shown that feeding of antigens to germfree mice results in a predominantly IgA type immune response. We completed an experiment in germfree mice in which various antigens will be fed during the latent period. Only a slightly increased incidence of plasmacytomas has developed in mice fed the Trichoderma extract. None of the tumors thus far has produced a myeloma protein that reacts with the extract.

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excellent crystals (see below).

2. Crystallographic analysis of the McPc603 phosphorylcholine binding protein.

In collaboration with Drs. S. Rudikoff, Eduardo Padlan, David Segal and David Davies we are solving the crystal structure and determining the amino acid sequence of the McPc603 pepsin fragment. A 4.5 Å electron density map has been obtained and the ligand binding site located. Dr. Rudikoff has completed the sequence to about position 46 on the V_K and 50 on the V_H .

3. Study of the IgC_H locus, linkage of IgV_H genes.

In the last several years, in collaboration with Miss Rose Lieberman (I:LCI-15c) we have produced myeloma-specific and allotypic antisera by immunization of other strains of mice with BALB/c myeloma proteins of the IgA and IgG classes. These antisera aid in the characterization of identifying specific gene products from the complex IgC_H locus. Understanding of the structure and regulation of this complex locus progresses at this time by comparative studies of homologous loci in other strains. Because plasmacytomas cannot be induced in other strains a complete collection of homogeneous proteins has not been available as a source of antigens for the preparation of allotypic antisera.

Congenic strains are now past the 20th backcross and may be considered to be essentially congenic with BALB/c except for the insertion of foreign heavy chain (IgC_H) complex: BALB/c x C57BL/KaIgC_H BC20 F₂(CB20) and BALB/c x ALIgC_H BC20 (AL-20). We have now completed the development of congenic strains of mice which provide a source of myeloma proteins directed by loci other than the one found in BALB/c.

A series of plasmacytomas have been induced in BALB/c x ALIgC_H BC9 F5 (CAL-9) by Mrs. Elizabeth Mushinski and these are being used as a source of myeloma proteins for a new series of allotypic studies in collaboration with Miss Rose Lieberman. We are continuing a search for all types of Ig from the C57BL IgC_H complex locus. In over 50 tumor studies we have been unable to recover a myeloma carrying the IgC_H (2) determinant. This result suggests that the gene controlling this protein is not regulated within the congenic host.

These strains are being used in collaborative studies with Dr. Al Nisonoff, University of Illinois, in searching for an idiotypic marker that is linked to allotype i.e. and IgV_H gene.

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These studies have been extended to include pathogen-free mice. Arrangements were made with Mr. Howard Bohner of the Lab Aids Division to establish a new pathogen-free BALB/c mouse strain contaminated with Lactobacillus, Streptococcus D and Bacteroides. These mice have been maintained continuously in tanks and have been given three injections of pristane (either of 1 or of 2 months).

Surprisingly the incidence of plasmacytomas in PF mice was very low, nearly that in GF mice. Several complications were encountered. ExPF mice proved difficult to maintain in dirty animal rooms which necessitated testing progeny of ExPF mice these have developed a high incidence of plasmacytomas. Essentially it is concluded, a changing mixed flora is an essential factor in plasmacytoma development. We further plan to test PF mice infected with E. coli for susceptibility.

II. Immunochemical Characterization of Antigen Binding Myeloma Proteins.

Genetic basis of diversity.

The mouse has been a rich source of homogeneous immunoglobulins with antigen binding activity. Some 21 proteins are under study in this laboratory that have binding activity for a variety of different polysaccharide moieties, phosphoryl choline and nitro-phenyl groups. For many of these the specific chemistry of the ligand is known but for others only an antigen has been identified and it becomes essential to find the chemical nature of the determinant group.

Groups of myelomaproteins, all of BALB/c origin, that bind the same hapten, provide an invaluable system for studying the molecular basis of diversity.

In collaboration with Dr. C.P.J. Glaudemans (A:LC-7) we are examining the binding affinities and specificities of 7 BALB/c myeloma proteins that bind Bl+6D galactan. We (Dr. S. Rudikoff, and Mrs. Mushinski) are determining the V-region sequences of the H and L chains and are comparing the proteins by antigenic (idiotypic) analysis. While it is clear all 7 of the proteins are different antigenically, they all nonetheless fall into the same, V_K subclass and probably also the same V_H subclasses. The complete sequences of these proteins is a long range goal but should provide valuable information on the basis of diversity. We are attempting to find more anti Bl+6D gal binding proteins in an attempt to find repeats. With Dr. Glaudemans, affinity site labelling compounds are being sought. One of the proteins in this series J539 gives

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Initial studies have revealed linkage of an antiarsonate idiotype to the ALIgC_H locus. This has now been confirmed in the CAL-9 and CAL-20 strains. The search for more markers in the V_H region continue.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3202

Title: Pathogenesis of plasma cell neoplasia in mice: immunochemical characterization of antigen-binding myeloma proteins; genetic basis of immunoglobulin diversity.

Principal Investigator: Michael Potter, Stuart Rudikoff, Elizabeth B.

Section: Immunochemistry

Mushinski

Lab/Branch: Lab. of Cell Biology, DCBD

Goal: 1. To establish the basic mechanisms in plasma cell tumor development

2. Determine the genetic basis of antibody diversity.

Approach 1. Plasmacytomas can be induced in the inbred BALB/c mouse by mineral oils and pure alkanes. The effect of the oil is to create an abnormal tissue microenvironment in which the tumors develop. We are studying the oil granuloma environment for its ability to stimulate the growth of plasmacytomas and its suppressive effects on host immunity to plasmacytomas. The role of viral and chemical initiators, and specific genes in plasmacytoma formation are being examined.

2. The antigen-binding myeloma proteins of mice are forms of homogeneous antibodies that can be used to analyze antibody structure. We isolate groups of myeloma proteins that bind the same hapten, determine their amino acid sequences, and crystal structure.

Progress: 1. The intraperitoneal injection of mineral oil (priming) creates an abnormal tissue environment long before the tumors appear. This has been demonstrated by successful growth of small numbers of plasmacytoma cells from a primary host, in the mineral oil conditioned peritoneum. Priming locally stimulates growth of and abrogates established immunity to an antigenic transplantable plasmacytoma. Abelson virus, infection in 5 o 8 weeks after mineral oil injection results in the rapid development of plasmacytomas and lymphosarcomas.

Significance for Cancer Research (NCP Objective	$\frac{2}{3}$	Approach	$\frac{3,4,5}{1,2}$
	$\frac{4}{1}$		

Level of Effort: Professional Man-Years: 2

Serial No. NCI-5553

1. Laboratory of Cell Biology
2. Immunochemistry Section
3. Bethesda, Md.

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Structure of mouse myeloma proteins with antibody activity

Previous Serial Number: Same

Principal Investigator: Dr. Stuart Rudikoff

Other Investigators: Dr. Michael Potter (NCI-5556)
Dr. Eduardo Padlan (NIAMDD-LMB-1)
Dr. David Segal (NIAMDD-LMB-1)
Dr. David Davies (NIAMDD-LMB-1)
Dr. Leroy Hood (Cal. Tech.)
Dr. Neil Glaudemans (NIAMD)

Man Years: Professional - 1

Project Description:

A series of mouse myeloma proteins with antigen binding activity have been developed in this laboratory by Dr. Michael Potter. We are currently studying two groups of these proteins in an effort to 1) determine the structure of the antibody combining site at the molecular level and 2) determine the degree of variability possible among immunoglobulins binding the same haptenic determinant.

1. Following our description last year of the crystallization of the pepsin Fab from the phosphorylcholine binding myeloma protein McPC 603, we have demonstrated that both the Fab in solution and the crystal bind hapten stoichiometrically. X-ray crystallographic studies have recently produced a 4.5 Å resolution map of the Fab in which several features of the immunoglobulin molecule are readily apparent. Two large globular domains representing the variable and constant portions of the molecule are seen. Each domain is divided into two halves which are presumably the light and heavy polypeptide chains. Using a heavy atom derivative of phosphorylcholine the antigen binding site has been located. The hapten is located in a large cleft between the light and heavy polypeptide chains at the tip of the variable domain. Data are now being collected for a 2.7 Å high resolution map.

We are currently determining the primary structure of the polypeptide chains. Using a Beckman Sequencer recently set up in our laboratory we have been able to determine the sequence for approximately 1/2 of the light chain and 1/3 of the heavy chain. The primary sequence determination plus

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the high resolution map will enable the construction of a molecular model. We have also recently crystallized the Fab from a galactan binding myeloma protein which we plan to use for a comparative study.

2. To approach the degree of variability among antibodies binding the same hapten we are currently studying two groups of proteins 1) those binding β -(1-6)-galactan and 2) those binding phosphorylcholine. We have recently identified and purified by affinity chromatography 6 galactan binding proteins. Each of these proteins possesses an individual antigenic specificity (idiotype) indicating primary structural differences. Preliminary sequence studies have shown that the light chains from all six proteins are identical for the first 23 residues. The heavy chains from 4 of the proteins are identical for 30 residues and the other 2 have single substitutions.

In contrast a study of 4 phosphorylcholine binding proteins has revealed a different pattern of variation. Two of the proteins, H8 and T15 are idiotypically identical and have the same primary structure through the first two hypervariable regions of the light chain. Two others, M 603 and M 167, which are idiotypically different have quite different light chain sequences from each other and from H8 and T15. The heavy chains from these proteins, however, are identical through the first hypervariable region with the exception of a single substitution in the M167 chain. Comparative sequence studies of these 2 groups of proteins are continuing.

It is felt that studies of this nature will enable the eventual in vitro design and synthesis of antibodies with directed specificities. These molecules have both diagnostic and therapeutic implications.

Relationship of research to National Cancer Plan:

Approach - 1
Objective - 6

Publications:

Voss, E. W., Rudikoff, S., and Sigel, M. M.: Comparative ligand binding properties of purified nurse shark natural antibodies to anti-2,4 dinitrophenyl antibodies from other species. J. Immunol. 107: 12, 1971.

Riesen, W., Nosedá, G., Rudikoff, S., and Potter, M.: An IgM Waldenström with anti-phosphorylcholine specificity. Colloquium on protides of the biological fluids. Bruges, 1972.

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Potter, M., Mushinski, E. B., Rudikoff, S., and Appella, E.: Antigen-binding myeloma proteins in mice: Groups of proteins that bind the same hapten. Third Int. Convoc. on Immunology. In press.

Rudikoff, S., Potter, M., Segal, D. M., Padlan, E. A., and Davies, D. R.: Crystals of phosphorylcholine-binding fab-fragments from mouse myeloma proteins: Preparation and X-ray analysis. Proc. Nat. Acad. Sci., 69: 3689, 1972.

Jolley, M. E., Rudikoff, S., Potter, M. and Glaudemans, C. P. J.: Special changes on binding oligosaccharides to murine IgA myeloma immunoglobulins. Biochemistry. In press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5553

Title: Structure of Mouse Myeloma Proteins with Antibody Activity

Principal Investigator: Stuart Rudikoff, Ph.D.

Section: Immunochemistry

Lab/Branch: Laboratory of Cell Biology, Division of Cancer Biology & Diagnosis

Goal: The goal of this project is to 1) ascertain the degree of genetic variability among antibodies with the same binding specificity and 2) define at the molecular level the antibody-antigen interaction.

Approach: We have approached these problems by studying mouse myeloma proteins which represent individual species of antibodies. Two large groups of antigen binding myeloma proteins have been described: 1) Five proteins which bind phosphorylcholine and 2) Six proteins which bind β -(1-6)-D-galactan. This study involves primarily a determination of the primary structure of these proteins using various sequencing techniques including protein fragmentation, peptide isolation and finally sequencing of peptides. At the same time we are in the process of determining the three dimensional structure of the antibody combining site. We have been able to approach this problem by successfully crystallizing the combining sites of two antigen binding myeloma proteins. X-ray crystallographic studies are currently under way to determine the molecular structure of the combining site as well as the molecular basis of the antibody-antigen interaction.

Progress: Studies over the past year have demonstrated that the crystallized Fab' of the phosphorylcholine binding myeloma protein, McPC 603 bind hapten stoichiometrically. Crystallographic studies have produced a 4.5 Å resolution model which delineates a number of features of the immunoglobulin molecule including 1) globular domains representing the variable and constant portions of the molecule and 2) the division of each domain into two halves representing the light and heavy polypeptide chains. Using a heavy atom derivative of phosphorylcholine we have located the hapten binding site in a large cleft between the light and heavy chains in the variable region. We have recently equipped a laboratory for sequence studies including an automated sequencer which has enabled us to determine approximately 1/2 of the light chain and 1/3 of the heavy chain sequence.

Significance for Cancer Research (NCP Objective 6 Approach 1)

It is felt that studies elucidating antibody structure at the molecular level may eventually lead to the in vitro design and synthesis of antibody molecules with pre-determined specificities. These molecules would be of extreme diagnostic as well as possibly therapeutic value.

Level of Effort: Professional Man-Years: One

Serial No. NCI-5541
1. Lab. of Cell Biology
2. Macromolecular Biology
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Binding of mycophenolic acid to serum albumins.

Previous Serial Number: None

Principal Investigator: Sidney Shifrin

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1/4
Professional: 1/4
Other:

Project Description:

Objectives: The purpose of this study was to examine the properties of mycophenolic acid (a drug reported to possess anti-viral and antitumor properties) and its ability to bind to serum albumin from a variety of species.

Methods Employed: Ultraviolet absorption spectroscopy, fluorescence, protein chemistry, disc gel electrophoresis, chromatography and radioactive labelling methods.

Major Findings: In alkaline solution mycophenolic acid has its long wavelength absorption band at 341 nm with an extinction coefficient of 6200. As the pH of the solution is lowered, the 341 nm band is lost and a new maximum appears at 304 nm. The band in the near ultraviolet at 250 nm remains relatively constant. The titration curve indicates that the chromophore which is the phenolic group of the drug has a pK of 7.5.

An examination of the fluorescence properties of the drug indicated that the ionized form had a weak fluorescence (quantum yield = .022) with maximum emission at 420 nm. A decrease in pH of the solution resulted in complete quenching of the compound. A titration curve of pH versus fluorescence intensity indicated

that the emitting group also has a pK of 7.5; therefore, quenching is directly associated with protonation of the phenolate ion. This behavior is in direct contrast with the fluorescence properties of phenol itself which is non-fluorescent in the ionized form and most fluorescent when the hydroxyl group is protonated.

In the presence of bovine serum albumin or human serum albumin there is a shift in the absorption maximum of mycophenolic acid from 341 to 345 nm accompanied by a decrease in absorbance. However, fluorescence is a more sensitive measure of changes in the microenvironment of the drug. The emission intensity of mycophenolic acid is enhanced nearly ten fold in the presence of human serum albumin and the maximum is shifted to 410 nm. Similar results were obtained with human prealbumin and bovine serum albumin but were not observed with other proteins having hydrophobic binding sites such as β -lactoglobulin, chymotrypsin, γ G-immunoglobulin or ovalbumin. Serum albumin from other species also showed enhancement. The other species included guinea pig, goat, dog, sheep, chicken, rabbit and mouse.

The fluorescence of the protein itself was quenched on addition of mycophenolic acid. The serum albumin was excited at 280 nm resulting in a fluorescence at 330 nm. The extent of quenching of this band and the intensity of the 410 nm fluorescence that results when mycophenolic acid is added to the albumin depends on the source of the protein. Human prealbumin contains 12 tryptophan residues per mole and 30% of its 330 nm fluorescence intensity is quenched when it is saturated with the drug. Only 1 mole of mycophenolic acid is bound to a mole of human prealbumin. Bovine serum albumin contains two tryptophanyl residues and saturation with the drug results in quenching of 60% of the 330 nm fluorescence. Bovine serum albumin binds 3.6 moles of mycophenolic acid. Human serum albumin contains 1 tryptophanyl residue per mole and its 330 nm fluorescence is almost completely quenched. The only fluorescence band remaining has its maximum at 305 nm which is due to the tyrosyl residue. Human serum albumin also binds 3.6 moles of mycophenolic acid. These results suggested that the drug binds in the vicinity of a tryptophanyl residue.

In order to determine whether the drug binds through ionic forces (i.e., the negatively charged carboxylate or phenolate of the drug to a positively charged amino acid side chain such as lysine in the albumin) we carried out a stepwise succinylation of human serum albumin. This replaced the positive charge on the lysine with a negative charge of the succinate. There did not appear to be any drastic conformational changes accompanying the succinylation.

Binding of mycophenolic acid as measured by quenching of 330 nm fluorescence of the protein or the appearance of 410 nm

fluorescence of the drug was markedly reduced when only 3.4 lysyl residues were succinylated. The potential for binding was almost totally lost when 8 succinyl residues were introduced. These results indicate that binding of the drug to the serum albumin occurs through electrostatic forces.

During the course of these studies we observed that the mycophenolic acid underwent drastic changes in the spectrum upon exposure to light. These changes were further enhanced in the presence of the serum albumins. Precautions should therefore be taken to protect solutions of mycophenolic acid from unnecessary exposure to light if it is to be used clinically.

Significance to Biomedical Research and the Program of the Institute: The specificity shown by the serum albumins for binding mycophenolic acid suggests that these proteins might serve as the carriers for this anti tumor agent. The suggestion that ionic forces are involved in this binding also leads to the recommendation that the phenolic residue should be methylated in order to prevent formation of the glucuronide which is the pathway through which the drug is rapidly excreted.

Proposed Course of the Project: The effect of chemical modifications of mycophenolic acid on binding to serum albumins is proposed.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5541

Title: Binding of An Antitumor Agent to Serum Proteins.

Principal Investigator: Dr. Sidney Shifrin

Section: Macromolecular Biology Section

Lab/Branch: Laboratory of Cell Biology, DCBD, NCI

Goal: To understand the mechanism by which mycophenolic acid is transported to the tumor and to increase its effectiveness.

Approach: Examination of the absorption and fluorescence properties of mycophenolic acid at various pH values and in the presence of a variety of cellular constituents. Chemical modification of cellular constituents such as serum proteins were carried out in order to determine the nature of the binding sites that are essential for the binding of this agent. We are also planning to modify the drug in such a way that it will not be rapidly excreted from the circulation and it will still retain its antitumor properties.

Progress: Mycophenolic acid was found to be fluorescent at pH values above 7.5. The fluorescence maximum at 420 nm is markedly enhanced concomitant with a shift to shorter wave lengths in the presence of some proteins. The proteins that have been found to produce this enhancement include: human prealbumin, bovine serum albumin and human serum albumin as well as the serum albumins from a variety of other sources. Other proteins that were examined that did not bind the drug included lactoglobulin, ovalbumin, chymotrypsin, immunoglobulin and glucuronidase. Thus it appears that the serum proteins bind this agent specifically and may function as the transport mechanism for getting the drug to the appropriate site.

Significance for Cancer Research (NCP Objective 6 Approach 1,2)

The present research is an attempt to understand the method by which mycophenolic acid is transported to the tumor and means by which its action could be enhanced.

Level of Effort: Professional Man-Years: 1/4 Man-years.

Serial No. NCI-5542
1. Lab. of Cell Biology
2. Macromolecular Biology
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Physicochemical properties of L-asparaginase
from Erwinia carotovora.

Previous Serial Number:

Principal Investigator: Sidney Shifrin

Other Investigators: Irwin Chaiken, NIAMDD

Cooperating Units: Laboratory of Chemical Biology, NIAMDD

Man Years:

Total:	1/2
Professional:	1/4
Other:	1/4

Project Description:

Objectives: The purpose of this investigation was to examine the chemical and physical properties of L-asparaginase from Erwinia carotovora and to compare them with the properties previously reported for the Escherichia coli enzyme. Since the enzyme from both sources is equally effective in the clinical treatment of leukemia, we wanted to examine structural features that the enzyme has in common.

Methods Employed: The enzyme was examined using automated amino acid analysis, chemical modification of amino acid side chains, absorption spectroscopy, ultraviolet difference spectroscopy, circular dichroic spectroscopy, fluorescence, polyacrylamide disc gel electrophoresis, sedimentation velocity ultracentrifugation and radioactive labelling techniques.

Major Findings: The amino acid analysis of L-asparaginase from Erwinia is considerably different than the E. coli enzyme. The former does not contain any tryptophanyl or cysteinyl residues and is a considerably more basic protein. Although it has nearly the same amount of tyrosyl residues as the E. coli enzyme, only 10% of them ionize with a normal pK compared with

30% in E. coli asparaginase.

The subunit structure of the Erwinia enzyme is quite stable and cannot be dissociated by 8 M urea or by 0.5% sodium dodecyl sulfate, conditions previously reported to completely dissociate E. coli asparaginase into its monomers. Slow dissociation takes place in 3.5 M guanidinium chloride as measured by ultraviolet difference spectroscopy. The rate is markedly enhanced on addition of alcohols with the increase being proportional to the length of the alcohol side chain. In the presence of the alcohols dissociation appears to take place by two distinct processes although these were not analyzed further in this study. Removal of the denaturants results in reassociation and recovery of more than 90% of the enzyme activity.

The lysyl groups of Erwinia asparaginase were reacted with radioactive succinic anhydride. Although nearly all of the positively charged lysines were converted to their negatively charged succinyl derivatives, there was no spontaneous dissociation. In addition succinylation did not destabilize the protein as measured by rate of dissociation in 3.5 M guanidinium chloride.

Hybrids were prepared from native and succinylated subunits of Erwinia L-asparaginase which showed 5 bands on polyacrylamide gels. These results indicated that the four subunits of this enzyme are identical.

Significance to Biomedical Research and the Program of the Institute: Although L-asparaginase from some sources are effective in the treatment of certain forms of leukemia, the enzyme isolated from other sources are of little clinical value. The present study is part of an effort to ascertain the molecular properties responsible for clinical effectiveness of this chemotherapeutic agent. The results obtained in this study suggest that the net charge of the enzyme is not an important factor nor does there appear to be any relationship between the ease with which the tetramer dissociates into its subunits and clinical effectiveness.

Proposed Course of the Project: L-Asparaginase from other sources are being sought in an effort to carry out studies similar to those reported here in order to achieve our goal.

Honors and Awards: None

Publications:

Shifrin, S., and Solis, B. G.: Reaction of N-acetylimidazole with L-asparaginase. Molecular Pharmacology 8: 561-564, 1972.

Loos, M., Vadlamudi, S., Meltzer, M., Shifrin, S., Borsos, T., and Goldin, A.: Detection of endotoxin in commercial L-asparaginase preparations by complement fixation and separation by chromatography. Cancer Res. 32: 2292-2296, 1972.

Shifrin, S., Solis, B. G., and Chaiken, I. M.: L-Asparaginase from Erwinia carotovora. Physicochemical properties of the native and succinylated enzyme. J. Biol. Chem. 248: 1973
In press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5542

Title: Physicochemical Properties of L-Asparaginase from Erwinia Carotovora.

Principal Investigator: Dr. Sidney Shifrin

Section: Macromolecular Biology Section

Lab/Branch: Laboratory of Cell Biology, DCBD, NCI

Goal: To determine the molecular features of L-asparaginase that are necessary for the antileukemic behavior of this chemotherapeutic agent.

Approach:

To elucidate the amino acid composition, net charge, subunit structure and the ease with which it is disrupted, and three-dimensional structure of asparaginases from several sources in an effort to determine those features that will help understand how this agent functions clinically.

Progress: L-Asparaginase from Erwinia carotovora differs markedly from the enzyme isolated from Escherichia coli which was previously studied in this laboratory. The amino acid compositions differ as well as the net charge on the protein. The Erwinia enzyme is considerably more basic. It has four identical subunits which are held together more tightly than the coli enzyme. Extensive succinylation does not cause spontaneous dissociation. These results indicate that the net charge on the protein nor the ease with which the subunits dissociate accounts for the effectiveness of this material.

Significance for Cancer Research (NCP Objective 6 Approach 1,2)

This research is an attempt to relate the molecular structure of asparaginase with its antileukemic activity.

Level of Effort: Professional Man-Years: 1/2 Man-Years

Serial No. NCI-5558-1
1. Laboratory of Cell Biology
2. Cellular & Tumor Immunology
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immune Response to Tumor Specific and Other Cell
Surface Antigens

Previous Serial Number: Same

Principal Investigator: Chou-Chik Ting, M.D.

Cooperating Unit: Laboratory of Biology, NCI; Bionetics
Research Laboratories, Contract PH 70-2207

Man Years:

Total:	2
Professional:	1
Other:	1

Project Description:

Objectives: The objectives of this project are: 1) To characterize the specificities of the tumor specific or tumor associated antigens in SV40-induced tumors, and in Friend virus-induced leukemia. 2) Expression of fetal antigens and tumor specific antigens in SV40 transformed cells. 3) Test for TSTA (tumor specific transplantation antigen).

Methods Employed: Tumor cells transformed by oncogenic viruses, chemicals, or unknown agents are kept in the tissue culture or as transplanted tumors in mice or rats. The isotopic antiglobulin technique (IAT) was used to perform serological studies. The TSTA was tested by immunorejection experiments.

Major Findings:

I. Characterization of the specificities of the tumor specific cell surface antigen (TSSA) and cross reactive antigens in SV40 tumors and Friend virus induced leukemia.

A. SV40 tumors.

1. Test of tumorigenicity and immunogenicity of mouse SV40 transformed cells: Most mouse SV40 transformed cells are not tumorigenic in immunocompetent mice, thus making

tumor transplantation experiments difficult to perform. We found that a BALB/c SV40 transformed cell line SVT₂ (obtained from Dr. Aaronson) was tumorigenic in immunocompetent mice (TD50 = 2×10^6) as well as in immunosuppressed mice (TD50 = 1×10^3). It was also immunosensitive to the immunization with SV40 TSTA; a 100-1000 fold protection could be obtained by immunization with SV40 transformed cells or SV40 (virus). Thus, SVT₂ cells will be very suitable to perform SV40 tumor transplantation studies in mice.

2. Serological analysis of the antigenic specificities: Two antigenic specificities could be demonstrated on SV40 tumor cells as tested by IAT. One was TSSA which was the same as we described before. The other was a cross reactive antigen which was shared by some non-SV40 tumor (e.g. polyoma Py 4198). Only the TSSA seemed to correlate with TSTA; e.g. immunization with Py 4198 gave no protection against the challenge with SV40 tumor SVT₂.

B. Friend virus induced leukemia.

We have studied the immune response of C57BL/6 (B6) mice to the syngeneic Friend virus induced leukemia FBL-3. This tumor cell line is very immunosensitive to the immunization with TSTA. Antisera could be produced by syngeneic immunization of B6 with FBL-3. When tested by IAT, it was found that three specificities could be defined; one was unique for FBL-3, the other was FMR, and the third was cross reactive antigen of fetal nature. Only the first two specificities seem to correlate with TSTA.

II. Expression of fetal antigens and tumor specific antigens in SV40 transformed cells.

A. Serological analysis of antigenic specificities.

We have previously reported that fetal antigens are expressed in tumor cells, but they are different from tumor specific antigens. We then further investigated the expression of fetal antigens and tumor specific antigens in various SV40 transformed cells. Testing by IAT, a common TSSA was found in various SV40 transformed cells and its expression correlated with function of the SV40 genome. In contrast, different fetal antigenic specificities are expressed in various SV40 transformed cells, and its expression was not directly related to the function of viral genome.

B. Tumor transplantation experiments.

When SVT₂ cells were used in the in vivo experiments, immunization with SV40 transformed cells or SV40 (virus)

produced 100-1000 fold protection, but immunization with fetal tissues gave no protection. These findings gave additional evidence that fetal antigens are different from tumor specific antigens.

III. Specificity of TSTA.

Various tumor associated antigens may be expressed in tumor cells. Some of them may function as transplantation antigen but may not be truly TSTA, e.g. fetal antigens. A test for the specificity of TSTA is needed to characterize these different antigens. We have explored the possibility that specific anti-tumor antibody may block the specific antigenic receptor sites of tumor cells, and thus render these cells to be non-immunogenic or less immunogenic to produce tumor immunity. We have performed experiments in the FBL-3 system. Our preliminary data indicated that by treating FBL-3 cells with anti-FBL-3 serum, it would indeed block the immunogenicity of these cells. Further experiments are needed to determine the specificity of this blocking effect.

Significance to Biomedical Research and the Program of the Institute: Testing by the IAT, both tumor specific and cross reactive antigens can be demonstrated in SV40 tumors and Friend virus-induced leukemia. Only the former (TSSA) seems to be correlated with TSTA, thus this assay may be used to characterize the TSTA, and to monitor the application of TSTA in immunoprophylaxis or immunotherapy. Although the cross reactive antigens do not seem to function as transplantation antigen in these two tumors, it still may be used for immunodiagnosis if these antigens are not present in normal adult tissues.

The correlation between the humoral antibody response to fetal antigens and the resistance to tumor challenge indicated that the effect of fetal antigen immunization on tumor growth was determined by the presence of fetal antigen (of transplantation type) on these tumor cells, and also by the host immune response to these antigens. The IAT may be used to evaluate the effectiveness of fetal antigens on immunoprophylaxis or immunotherapy of some tumors.

The term TSTA is originally defined by in vivo immunorejection experiments; but it has been mistakenly used in many laboratories with other tumor-associated antigens, or even with tissue or organ specific antigens. If the specificity of TSTA can be tested, then this important issue can be clarified.

Proposed Course of Projects:

I. Test the specificity of TSTA.

We have prepared specific antisera reacting with the TSSA of SV40 tumors and Friend virus-induced leukemia. If these sera specifically block the immunogenicity of the respective cells in vivo, then the specificity of TSTA can be tested by reacting the tumor cells with specific antisera, and test the effect of antisera on its immunogenicity. Controls will include using various unrelated sera, and unrelated tumor cells. Furthermore, the specific antisera can be absorbed with various tumor cells or normal tissue cells.

II. Cell-mediated immunity (CMI) in vitro.

We plan to use the ⁵¹Cr lymphocyte cytotoxicity test and ¹²⁵IUDR-cytotoxicity test to study the CMI of Friend virus-induced leukemia (FBL-3), and SV40 tumors, respectively. We want to concentrate on the specificity of these reactions, and the blocking of CMI by antisera.

III. Mouse mammary tumor (MMT).

We originally planned to study the tumor associated antigens in human breast carcinoma. After some initial attempts, many problems were encountered: difficult to obtain tumor cells for testing, presence of reactivity in normal individuals, etc. Therefore, we decided to use MMT as a model to study some basic questions.

A. Cross immunity (in vivo) between various MMT: (includes tumors which contain MTV or free of MTV). Different MMT will be used to immunize C3Hf, then challenge with C3H MMT.

B. Humoral antibody response to MMT: IAT will be used to study the kinetics of antibody response in tumor-free and tumor-bearing animals. The specificity of the antisera will be checked. We are most interested in the non-virus-associated tumor-specific cell surface antigen in various MMT.

C. CMI in vitro: ¹²⁵IUDR assay will be used. We shall study the specificity of the reactions, blocking effect of antisera, and its correlation with tumor growth.

IV. Detection of anti-tumor antibody in tumor bearing animals.

Testing by IAT, antibodies reacting with TSSA or tumor associated antigens can be detected in tumor-bearing animals

with SV40 tumors, Friend virus-induced leukemia (FBL-3) or Rauscher virus-induced leukemia (RBL-5). We plan to study the kinetics of antibody response in relation to the dose of tumor inoculation, and to tumor growth. This may offer a model for establishing an immunodiagnostic test of tumors.

Publications:

1. Ting, C.C., Lavrin, D.H., Shiu, G., and Herberman, R.B.: Expression of fetal antigens in tumor cells. Proc. Nat. Acad. Sci. U.S.A., 69:1664-1668, 1972.
2. Ting, C.C., Lavrin, D.H., and Herberman, R.B.: Antibodies to fetal antigens. In Proc. Second Conf. and Workshop on Embryonic and Fetal Antigens in Cancer. N.G. Anderson and J.H. Coggin, Jr. (eds.), 1972, pp. 111-116.
3. Rosenberg, E.B., Hill, J., Ferarri, A., Herberman, R.B., Tinc, C.C., Mann, D.L., and Fahey, J.: Prolonged survival of tumor allograft in mice pretreated with soluble transplantation antigens. J. Nat. Cancer Inst. (in press).
4. Ting, C.C., Ortaldo, J.R., and Herberman, R.B.: Expression of fetal antigens and tumor specific antigens in SV40 transformed cells. I. Serological analysis of the antigenic specificities. Int. J. Cancer (in press).
5. Ting, C.C., Rodrigues, D., and Herberman, R.B.: Expression of fetal antigens and tumor specific antigens in SV40 transformed cells. II. Tumor transplantation studies. Int. J. Cancer (in press).

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-5558-1

Title: Immune Response to Tumor Specific and Other Cell Surface Antigens

Principal Investigator: Chou-Chik Ting, M.D.

Section: Cellular and Tumor Immunology Section

Lab/Branch: Laboratory of Cell Biology

Goal: To study immune response to tumor specific and other cell surface antigens.

Approach:

1. Serological characterization of tumor associated antigens: Antisera were prepared by immunization of mice with syngeneic SV40 tumor (SVT₂ in BALB/c) or Friend virus-induced leukemia (FBL-3 in C57BL/6). The isotopic antiglobulin technique (IAT) was used to perform serological testing.

2. Immune response to fetal antigens: Serological testing (IAT) and in vivo immunorejection experiments were performed to study the immune response to fetal antigens in different strains of mice.

Progress:

1. Tumor specific cell surface antigen (TSSA) and cross reactive antigens can be demonstrated in SV40 tumors and Friend virus-induced leukemia by serological testing. Only the TSSA seems to be correlated with tumor specific transplantation antigen (TSTA). The assay for TSSA may be useful to monitor immunoprophylaxis or immunotherapy of tumors.

2. The fetal antigens expressed in various SV40 transformed cells were not directly related to the function of SV40 genome. The fetal antigens were apparently different from SV40 tumor specific antigens. Immune response to fetal antigens varied markedly in different strains of mice.

Significance for Cancer Research (NCP Objective 4 Approach 2)

Level of Effort: Professional Man-Years: 1

Serial No. NCI-4833

1. Laboratory of Cell Biology
2. Ultrastructural Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electron microscope, biological and biochemical studies of normal, malignant and virus infected cells and cell fractions

Previous Serial Number: Same

Principal Investigators: Dr. Nelson A. Wivel
Dr. Stringner Sue Yang

Other Investigators: Dr. D. V. Ablashi (NCI-4834)
Dr. E. Kuff (NCI-366)
Dr. G. Smith (NCI-3526)
Dr. E. B. Thompson (NCI-5210)

Man Years: Total - 3.00
Professional - 2.00
Other - 1.00

Project Description:

Objectives:

The Ultrastructural Biology Section is concerned with the study of various factors which influence cell differentiation and malignant transformation. Particular emphasis is given to systems in which viruses represent the transforming agent. The projects undertaken require the correlation of data from various disciplines such as electron microscopy, virology, biochemistry, and immunology.

Methods Employed:

The principal methods involve the visualization of appropriate selected biological materials in the electron microscope, the use of tissue culture for propagation of viruses, the use of experimental animals for propagation of tumors, the use of differential and density gradient centrifugation to isolate cell and virus fractions, the use of radioisotopes to label viral nucleic acids, and the detection of viral antigens by a variety of immunological techniques.

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Major Findings:

1. Intracisternal A particles. As a result of a continuing study of intracisternal A particles, several important findings have developed. In brief, these particles are virus-like structures which are found in a variety of normal and neoplastic mouse tissues as well as tumors of the rat, gerbil, guinea pig and man. They are antigenically distinct from other known oncornaviruses (B particles and C particles). During the past year it has been established that there is a 60-70S RNA associated with intracisternal A particles. (See Dr. Yang's report). In addition it has been observed that there is an enzyme activity intrinsic to the particles which has the characteristics of a reverse transcriptase. These two characteristics are both consistent with the hypothesis that intracisternal A particles represent some expression of a viral genome. A study of the structural organization of these A particles has recently been published in collaboration with Dr. E. Kuff, and indicates that the outer of the two concentric shells is largely, but not entirely, susceptible to treatment with a variety of nonionic detergents. The inner shell is resistant to nonionic detergents and is stabilized against solubilization by sodium dodecyl sulfate as a result of disulfide bonding between its constituent proteins. It is now established that the major structural protein (molecular weight of app. 70,000) is contained in the inner shell, while a protein component with a molecular weight of about 80,000 may be preferentially concentrated in sites of firm attachment between the inner and outer shells. There are multiple minor protein components as determined by polyacrylamide gel electrophoresis and these components differ proportionally when A particles are derived from different tumor sources; these variations have the potential of serving as markers to be used in the analysis of the genetic determination of A particle expression.

2. Intracytoplasmic A particles. In a continuing collaboration with Dr. G. Smith, the properties of intracytoplasmic A particles are being studied. A major thrust of this investigative effort is to determine if this type of A particle is a precursor of the B particle or mammary tumor virus. An improved more rapid method for isolation has been developed and a detailed study of structural proteins indicates that there are three major bands when compared to the B particles which possess five major bands, as seen by gel electrophoresis. These three A particle bands all differ in molecular weight from the five major bands in the B particle. In view of this information, it is apparent that there are significant structural disparities between cytoplasmic A particles and B particles; the full implications of these findings remain to be determined. Further efforts to characterize isolated intracytoplasmic A particles are concentrated in two areas, the study of intrinsic nucleic acids (Dr. Smith) and the study of DNA polymerases (this laboratory); given this data we should be able to determine if intracytoplasmic A particles represent the nucleoprotein cores of mouse mammary tumor virus.

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Dr. Yang:

3. Intracisternal A particle RNA. The existence of an endogenous high molecular weight, 60-70S, RNA in intracisternal A particles derived from N-18 neuroblastoma cells has been demonstrated by a combination of radiochemical and physicochemical techniques. This 60-70S RNA is sensitive to ribonuclease digestion and alkali treatment, but is resistant to pronase treatment; this RNA consisted of subunits of 35S RNA. Other RNA species associated with intracisternal A particles are 4S, 8-12S and possibly 18S. The 60-70S, 35S, and 8-12S RNAs are being studied with regard to their functional significance, i.e., their templating activity for both transcription and translation; accordingly, the 4S RNA is being studied for its amino acid transfer activity.

4. Reverse Transcriptase and anti-tumor inhibitors. Previous work pointed to the possible use of reverse transcriptase (RNA-dependent DNA polymerase) as a diagnostic tool for viral carcinogenesis in tumors of murine and simian type. It is therefore important to evaluate some of the biochemical properties of reverse transcriptases from various tumor viruses and to correlate them with infectivity. A comparative study on heat inactivation was done by comparing the half life ($t_{1/2}$) of reverse transcriptases from various tumor viruses; this showed that the $t_{1/2}$ of reverse transcriptases from murine leukemia and sarcoma viruses is much shorter than those of feline leukemia and sarcoma viruses. These results correlated closely with the $t_{1/2}$ of the infectivity ability of the respective viruses on host cells. Results suggested that the presence of an active reverse transcriptase is an essential factor, if not the sole one, for cell infectivity. Some selected rifamycin derivatives have been demonstrated as potential inhibitors of cell transformation by murine sarcoma virus and of viral reverse transcriptase. They therefore lend themselves as potent anti-tumor drugs. Currently the mechanism of inhibitory action by these inhibitors on reverse transcriptase is under investigation. It is found that dimethyl benzyl rifampicin, an extremely potent inhibitor binds with reverse transcriptase as well as the viral core, whereas rifampicin, which does not inhibit reverse transcriptase, fails to bind with either the enzyme or the viral core. Specificity of binding to the enzyme protein and the viral core was therefore demonstrated with the potential anti-tumor inhibitor, dimethyl benzyl rifampicin.

5. A novel entity associated with a simian lymphoma cell line (AA). A tissue culture cell line (AA), obtained from Dr. Ablashi, was established earlier from a malignant lymphoma induced by Herpesvirus saimiri (HVS) in an owl monkey. HVS antigen was detected through the first four *in vitro* passages although HVS production was not observed. Chromosome aberration became apparent after the seventh passage. After the ninth passage, an enzymatic activity analogous to the reverse transcriptase of oncornaviruses was detected both in the AA cells and in the culture medium. It possesses major biochemical properties that meet the criteria of reverse transcriptase. When the partic-

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ulates in the tissue culture medium were concentrated and subjected to isopycnic density gradient centrifugation, the majority of the reverse transcriptase activity was found in the fractions with a density of 1.150-1.165 g/cm³. In addition the material in this same density region was shown to incorporate ³H-uridine. Although no virus-like particles have yet been detected ultrastructurally, the present biochemical evidence strongly suggests that an oncogenic RNA virus may be present, albeit in low titer. The relationship of this entity to HVS poses interesting and significant questions, all of which are worthy of further pursuit.

6. Induction of specific phenylalanyl transfer ribonucleic acid (phe-tRNA) by dexamethasone phosphate in Hepatoma Tissue Culture (HTC) cells. In collaboration with Dr. E. B. Thompson and Dr. M. Lippman we have found that a specific increase in phenylalanine-chargeable tRNA can be induced by the steroid dexamethasone phosphate in HTC cells. The increase in phenylalanine acceptor activity in steroid treated cells was not accompanied by changes in the intracellular phenylalanine pool, phenylalanine transport or gross increase in other species of tRNA and was dependent on de novo RNA synthesis. Furthermore, this increase in phenylalanine acceptor activity following steroid treatment seems to be coordinate with but separable from the induction of tyrosine aminotransferase. In vitro analyses also demonstrated a net increase in phe-tRNA in steroid treated cells as compared with non-treated cells and that this net increase is accounted for by the increase in one of the two iso-accepting forms of phe-tRNA as resolved by reverse phase chromatography.

Relationship of research to National Cancer Plan:

Project Area - 10

Approach Elements - 2

Approach - 4

Objective - 1

Publications:

Smith, G. H. and Wivel, N. A.: Isolation and partial characterization of of intracytoplasmic A particles. In Mouriquand, J. (ed.): Seventh International Colloquium on Mammary Tumors. 1972, pp. 157-162.

Wivel, N. A., Lueders, K. K., and Kuff, E. L.: Structural organization of murine intracisternal A particles. J. of Virology 11: 329-335, 1973.

Yang, S. S. and Wivel, N. A.: Analysis of high molecular weight RNA associated with intracisternal A particles. J. of Virology 11: 287-298, 1973.

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Ablashi, D. V., Loeb, W. F., Armstrong, G. R., Yang, S. S., Valerio, M. G., and Adamson, R. H.: Herpesvirus saimiri induced lymphoma and the DNA polymerases of the lymphoma derived cell line and Herpesvirus saimiri. J. Medical Primatology 3: 1-5, 1973.

Yang, S. S., Herrera, F., Smith, R. G., Ting, R. C., Gallo, R. C.: Analysis of 204 rifamycin derivatives: Some potent and selective inhibitors of Rauscher (murine) leukemia virus reverse transcriptase and of DNA polymerases from human normal and leukemic lymphoblasts. J. Nat. Cancer Inst. 49: 7-25, 1972.

Ting, R. C., Yang, S. S., and Gallo, R. C.: Reverse transcriptase, RNA tumor virus transformation and derivatives of rifamycin SV. Nature 236: 163-166, 1972.

Gallo, R. C., Smith, R. G., Whang-Peng, J., Ting, R. C., Yang, S. S., and Abrell, J. W. RNA tumor viruses, DNA polymerases, and oncogenesis: some selective effects of rifampicin derivatives. Medicine 51: 159-168, 1972.

Gallo, R. C., Abrell, J. W., Robert, M.S., Yang, S. S., and Smith, R. G.: Monkey tumor virus, avian myeloblastosis virus, and Rauscher leukemia virus and its response to rifamycin derivatives. J. Nat. Cancer Inst. 48: 1185-1189, 1972.

Smith, G. H., and Wivel, N. A.: Intracytoplasmic A particles: Mouse mammary tumor virus nucleoprotein cores? J. of Virology. In Press.

Wivel, N. A., and Yang, S. S.: Studies of nucleic acid metabolism in murine intracisternal A particles. Biophys. & Biochem. Res. Commun. In Press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-4833

Title: Electron microscope, biological and biochemical studies of normal, malignant and virus infected cells and cell fractions

Principal Investigator: Dr. Nelson A. Wivel Dr. Stringner S. Yang

Section: Ultrastructural Biology Section

Lab/Branch: Laboratory of Cell Biology

Goal: Our major research goal is to precisely identify viral replicative intermediates related to known oncogenic RNA viruses. Such data about partial viral gene expression may aid in the early diagnosis of the neoplastic state.

We are pursuing a multidisciplinary approach with electron microscopy and biochemical and biological analyses for an accurate identification of intracellular viral replicative intermediates. Ultrastructural guidelines accepted for known oncogenic viruses are being followed. Biochemically, the following analyses used for oncogenic RNA viruses are serving as our reference points: (1) high molecular weight RNA and its subunit structure; (2) reverse transcriptase and the transcriptional process; (3) DNA-RNA and DNA-DNA competitive hybridization studies using the product of reverse transcriptase with viral RNA and cellular DNA; (4) viral RNA and the translational process; and (5) the translational products and their homology with cellular components. The first three biochemical approaches along with ultrastructural analysis will serve to identify a viral replicative intermediate, whereas the last two will help to elucidate the process of viral genesis. Lastly, the process of viral infectivity and the mechanism involved in viral genome expression in cells will be studied by chemical induction, cocultivation and cell fusion experiments.

Progress:

As an ongoing project we have been studying murine intracisternal A particles and have recently established that they contain an intrinsic 60-70S RNA and an enzyme activity with characteristics of reverse transcriptase. Both of these findings suggest that the particles may well represent a viral genome.

Significance for Cancer Research (NCP Objective 1 Approach 4)

Our ultimate goal is to determine the relationship of these intracellular A particles to malignant transformation, both in the murine model system and in man, since they have been described in human tumors. Information derived from these studies will help to explain what forms of viral gene expression are pertinent to the induction and maintenance of the neoplastic state.

Level of Effort: Professional Man-Years: 2

ANNUAL REPORT SUMMARY

SURGERY BRANCH

NATIONAL CANCER INSTITUTE

April 1, 1972 - March 31, 1973

The Surgery Branch has continued the dual responsibility of providing surgical consultative and operative assistance to the multidisciplinary intramural clinical units of the National Institutes of Health while developing its own laboratory and clinical research programs. Approximately 60% of the Surgery Branch staff's activities are consumed by clinical responsibilities. During the past 12 months, 669 individually submitted surgical consultations were received, in addition to supervising 1054 requests for consultative services from extramural consultant sources. These figures include only the original consultative requests and are not inclusive of the tremendously larger total number of visits that were repetitively made to follow up the surgical consultative recommendations or surgery itself. Surgical procedures in the operating room were performed upon 724 patients during the past year, of which 307 were performed as part of the specific clinical research program of the Surgery Branch itself.

The ongoing clinical investigative program of the Branch can be categorized into four areas of chief interest: Cancer of the soft tissue pelvic structures, head and neck area, soft tissue and bone sarcomas, and melanomas. Each of these areas of interest have incorporated into them an ever increasing therapy trend. There is expanded programming with radiotherapy, chemotherapy (cytotoxic and anticoagulative agents), cryotherapy, and a surge of collaborative monitoring through immunological surveillance. Pelvic Structures: The role of radical surgery in the successful management of patients with radiation recurrent cancer of the cervix and vagina has been well established. A 41.2% actuarial five-year survival has been obtained in 115 patients with recurrent treatment failure disease, and in 94 patients with locally advanced primary cancer the survival has been 65%. A most significant aspect of this program has been the refinement of techniques which allow a safe and satisfactory means of diverting the urinary stream through the means of using either small or large bowel isolated conduit. Following pelvic exenteration, rehabilitative procedures such as vaginal plastics continue to be successfully utilized in selected patients. The few patients who are presently being admitted into this program, which we are rapidly phasing out, are being done so in order to complete some of the surgical-physiological monitoring studies that have been of long term interest to the Branch. Head and Neck: Local disease recurrence subsequent to treatment of cancers of the head and neck area continues to be an enigma. Based upon new evidence indicating that the presence of an isolated mass of tumor cells which might remain subsequent to conventional head and neck therapy might be highly sensitive to adjuvant therapy regimes, the Methotrexate study has been expanded. Preoperative use of this agent has shown a 72% primary tumor response rate without altering surgical morbidity. This is in contrast to the morbidity which has been repetitively demonstrated in adjuvant radiotherapy or radical surgery studies. Because of the morbidity potentially associated with the intracranial transfacial approach to paranasal sinus cancer, adjuvant studies

have not yet been incorporated into this longstanding study program. Fifty-four patients have now undergone the en bloc resection of their paranasal sinuses through this unique operative approach with an actuarial survival of 48% and a five-year survival of 58%. The use of extended deltopectoral full thickness skin flaps continues under tense investigation as the modality which best allows functional and cosmetic reconstruction of the head and neck area subsequent to cancer extirpation. New innovations in total mandibular replacement have shown early success due in part to the ability to adequately protect the mandibular foreign body from predisposing to secondary infection and fistulization. The uniquely innovative study of taste and smell in patients who have undergone cancer therapy of the head and neck area continues to be carried on aggressively in collaboration with the National Heart and Lung Institute. While studying the mechanisms of taste and smell it has become most apparent that there are compensatory alterations in these functions following local and in some areas regional excision of what has been thought to be the most sensitive of taste and smell areas. Sarcomas: Soft tissue and particularly bone sarcomas remain an enigma to the cancer therapists. The Branch experience with a limited number of each of the particular sarcomas indicates that the five-year survival following amputative surgery, with and without adjuvant therapy of several modes, is approximately 16% with osteogenic sarcomas and 51% with chondrosarcomas. The usual fibrosarcoma, rhabdomyosarcoma, and liposarcoma patient has a survival of approximately 58%. It has been demonstrated that hemipelvectomy can safely be performed either by the conventional technique or by the lateral position technique developed by the Branch, and offer satisfactory cure rates. These patients can also be functionally rehabilitated through the cooperative efforts of the physical and occupational therapist. Because of the propensity of these soft tissue and bone sarcomas to early metastases, these patients, all new admissions, are entered into an anticoagulative study whereby surgery is carried out under low dose maintenance anticoagulation. Preliminary 12-month evaluation of these study patients indicates that there may be a decreased propensity to metastases. Postoperative morbidity associated with this anticoagulative study has been shown to be directly related to the difficulty in maintaining adequately monitored levels of anticoagulation. This program has been expanded to include the addition of Adriamycin to the long-term postoperative management of those patients with high risk metastases-prone sarcomas. Melanomas: In achieving local control of the cutaneous lesions, results of standard wide-margin surgical excision are satisfactory, with a recurrence rate of less than 7%. Failure to control distant metastatic disease, however, is the primary cause of the overall mortality of 54% in five years. In order to more accurately determine the most suitable indicated for the treatment of melanoma, more accurate and comprehensive modes of staging are being developed. Subsequent to the usual clinical staging techniques, patients are receiving preliminary intra-abdominal exploration with multiple lymph node and organ body biopsies. Appraisal of the past experience of 262 patients treated for melanoma indicates that a five-year survival of 46% is directly related to the type of melanoma that is treated and the steps of invasion of the lesion, size of the primary pigmented skin abnormality, and the presence or absence of regional lymph node disease.

The Breast Oncology Section of the Branch has joined forces with the Medical Breast Unit in a Breast Tumor Section for treatment of breast cancer with

combined disciplines and facilities for breast cancer. Over the entire range of breast disease from early detection of suspicious breast lesions in a screening clinic, to primary surgical therapy to be carried out by the Branch, to adjuvant chemotherapy programs, pre- and postmenopausal first recurrent chemotherapy programs, and metastatic disease protocols, the Branch will be involved in the diagnosis, decision making, and therapy of breast cancer patients in collaboration with the surgeons' chemotherapy counterparts in the Medical Breast Division of the Breast Tumor Service. The Branch is involved also in aspiration, biopsy, primary surgical treatment, endocrine ablative procedures, and staging for all the patients entered into the service. The program is being rapidly expanded and developed along the lines of already prepared randomized protocols to be of maximum benefit to the patients in combination therapy and to achieve optimum yield of computerized data for the controlled studies jointly sponsored by the Surgery and Medicine Branches.

In collaboration with the Cytopathology Department, fine needle (22 gauge) aspiration biopsy for the cytologic diagnosis of solid tumors is being evaluated. Findings of these studies have been reported to the American College of Surgeons meeting in October 1972 and at a workshop for cytopathologists conducted in cooperation with the Cytopathology Department. Results to date indicate that diagnoses of cancer obtained on the basis of fine needle aspiration have been confirmed in all cases in subsequent histologic examination of excised tissue. A negative report, however, does not rule out malignancy. No untoward reactions or complications have been associated with this procedure. Use of the procedure has been extended from aspiration of subcutaneous and superficial lesions in all areas of the body to transrectal and transvaginal aspiration of deep pelvic masses and transantral aspiration of paranasal sinus tumors.

Studies carried out on patients with ileal conduits constructed for urinary diversion following urinary bladder resection for genitourinary tract carcinoma have established the normal cytology of ileal conduit urine. Abnormal ileal conduit urine cytology found in four patients has permitted detection of recurrent cancer involving the ileal conduit or upper urinary tract. Radioisotope renography studies in the evaluation of patients with ileal conduits have been shown to be a valuable aid in the follow-up of patients after radical pelvic surgery. Studies demonstrated the importance of the sitting position when these patients are evaluated by renography to avoid the false obstructive pattern frequently seen when the examination is carried out in the customary supine position. The renogram has been found to be a sensitive indicator of urinary tract obstruction which correlates well with excretory urography. Accordingly, long-term follow-up of patients following pelvic surgery can best be accomplished with periodic renograms and flat plates of the abdomen, rather than with the more complex excretory urography.

Paralleling the findings of other investigators in patients with lymphoid tumors, *in vitro* lymphocyte reactivity to phytohemagglutinin (PHA) has been shown to correlate with prognosis in patients with solid malignancies. Thus far, 94 patients have been followed for over three years after surgical exploration of their tumors and it is now possible to correlate their preoperative lymphocyte reactivity with subsequent clinical course. The lymphocyte

reactivity in autologous sera of each patient was compared with the mean age-correlated reactivity ± 1 standard deviation (SD) of 120 normal controls. In each group of cancer patients there was a larger percent, compared with controls, of individuals with lymphocyte reactivity below 1 SD of the mean reactivity of the controls. In the cured patient group, however, the percent with this low level of reactivity was smaller than in the inoperable or recurrent tumor groups. Also in the cured patient group a larger percent, compared with the controls and the inoperable and recurrent tumor groups, had lymphocyte reactivity that exceeded 1 SD of the mean reactivity of the controls. Patients with inoperable tumors and recurrent tumors had similar distributions of lymphocyte reactivity. The results show that relatively high preoperative lymphocyte reactivity correlates with a favorable prognosis after definitive surgery for cancer, but relatively low lymphocyte reactivity per se does not indicate a poor prognosis.

Lymphocyte reactivity to PHA is presently determined in AB pooled sera to allow separate determination of reactivity of lymphocytes and the effects of patient sera on normal lymphocyte reactivity. Thus far, 183 patients with non-lymphoid tumors have been studied by this method. Cells and sera from 384 healthy volunteers provided age-correlated control data. Impaired lymphocyte reactivity was demonstrated in patients with squamous carcinomas, sarcomas, and melanomas, but patients with adenocarcinomas show no defects. Serum suppressants of lymphocyte reactivity were demonstrated in patients with squamous carcinomas and sarcomas. Similar incidences of abnormalities were present in patients with primary tumors only and those with metastases. These data indicate that this investigative system assays early and tumor-related aspects of the host-tumor relationship and, therefore, are of potential usefulness in the study of factors associated with the induction of cancer and in monitoring the effect of tumor treatment.

Recently the Branch has devised a method of quantitating dinitrochlorobenzene (DNCB) contact sensitivity. With this technique, patients are scored with a quantitative scheme that distinguishes levels of positive reactivity in addition to anergy. With this method, sensitivity to DNCB was studied in 143 healthy volunteers between the ages of 20 and 80 years. Among these subjects, 96.5% had a spontaneous flare to DNCB. Thus far, over 200 cancer patients had a low incidence of spontaneous flare reactions (32%), a higher incidence of impaired reactivity (29%) and a higher incidence of anergy (30%) compared with the control population. While abnormal reactivity occurred in all histologic types of tumors studied, a significant relationship was shown between tumor histology and the distribution of abnormalities. Reactivity was most abnormal in patients with squamous carcinomas, less in patients with melanomas and adenocarcinomas, and least in patients with sarcomas. With the exception of patients with sarcomas, the incidence of impaired cellular reactivity was similar in patients with clinically localized tumors and those with disseminated cancer.

Correlation of DNCB contact sensitivity with in vitro lymphocyte reactivity to PHA in the preoperative patients shows that mean PHA reactivity increases with each level of DNCB reactivity from 0 to 4+. Furthermore, this correlation was present with all histologic types of solid tumors. These correlations indicate

that the use of both assays together should have increased value in quantitating cellular immune reactivity of cancer patients.

To determine the usefulness of in vitro lymphocyte reactivity to PHA and DNCB contact sensitivity combined as determinants of the tumor status of cancer patients, cellular immune competence was determined with these assays in over 80 postoperative patients considered clinically free of tumor and compared with that of the preoperative patients and normal controls. The immune reactivity of the cured cancer patients correlated with the histology of the tumor previously excised. Patients cured of squamous carcinomas had high incidences of impaired lymphocyte reactivity and DNCB contact sensitivity similar to those found in preoperative patients. Patients with sarcomas, melanomas, and adenocarcinomas, however, had lymphocyte reactivity higher than their preoperative counterparts and normal subjects, and did not display the abnormalities of DNCB contact sensitivity present in comparable preoperative groups. Among patients cured of cancer, as in preoperative patients, only those with squamous carcinomas and sarcomas had serum suppressants of lymphocyte reactivity. The persisting high incidence of impaired cellular immunity in patients cured of squamous carcinomas indicates the need for investigation of genetic and environmental factors which may be responsible for the defects and the determination of their relation to tumor induction. The intact cellular immune competence of patients clinically cured of sarcomas, melanomas, and adenocarcinomas indicates that patients treated for tumors of these histologic types who display abnormalities by the assays used may be suspect of having residual tumor.

The possibility that the persisting immune defects in certain cured cancer patients are at least in part due to genetic factors has led to the analysis of HLA profiles in cancer patients. Thus far, a significantly increased frequency of HLA-8 has been demonstrated in 103 patients with squamous cancer (41% vs. 18% in 250 controls). Over 100 patients with solid tumors of other histologic types had statistically similar HLA-8 frequencies as in controls. The presence of HLA-8 correlates with a lower PHA-induced lymphocyte reactivity in both normal controls and cancer patients. These data are the first evidence for a correlation of genetic factors with the presence of cancer and with immune reactivity in humans.

A method of quantitating peripheral blood monocyte function has been developed that utilizes latex particle phagocytosis and nitroblue tetrazolium reduction. Among over 110 preoperative cancer patients studied, a high percentage have defective monocyte function when compared with over 100 normal controls and with cured cancer patients. These data support other evidence for an important role of the macrophage in the host response to malignancy and suggest that this assay may be useful as a monitor of the effects of tumor treatment and detector of occult residual tumor after treatment.

The demonstrations of the value of total parenteral nutrition in chronically ill or malnourished patients has led to the adoption of a total parenteral nutrition registrar and the standardization of this new technique to surgical patients who have undergone major abdominal operations. As a result, only one non-infectious complication of subclavian catheter insertion has occurred since July 1972. Infectious complications, which occurred in significant incidence before July 1972 with several episodes of candidemia and bacterial septicemias,

have not occurred since then. In addition, a thorough metabolic study with nitrogen balances and electrolyte balances has been conducted on each patient, which allows the study of the metabolic changes with starvation and parenteral nutrition, as well as making hyperalimentation safer for critically ill patients.

The Branch has investigated the relationship of surgical stress and coagulation activation upon (1) the development of postoperative venous thrombosis; (2) the interaction of the coagulation mechanism and kinin generation in the production of the postoperative inflammatory response; and (3) the development of metastases. Template bleeding time, glass bead adhesion, and platelet factor 3 assays demonstrate in vivo and in vitro that two periods of platelet hyperactivity exist, namely on the second and eighth to tenth postoperative days. This correlates with the period of greatest risk of postoperative thromboembolic complications. The non-invasive bedside technique of impedance rheography has been used to detect both symptomatic and asymptomatic venous occlusions, and in the upper extremities to detect occlusions secondary to central venous catheterization. The data show that Hageman factor is activated intraoperatively within the first two hours of the onset of surgery. This observation supports the concept that the intrinsic coagulation system is activated by surgical stress, but places the time of activation earlier than previously thought, at the time of onset of trauma. Assays of bradykinin activity in biological fluid are difficult to interpret since the naturally occurring kininases so greatly abbreviate the life of generated bradykinin. Significant activation and utilization of the kinin precursors, pre-kallikrein and kininogen, begins intraoperatively and peaks on about the first postoperative day. These studies collectively are consistent with clinical observations of the appearance of postoperative inflammation and coagulation changes, imply a central role for Hageman factor in these phenomena, and may provide a basis for pharmacologic manipulation.

Previous work by the Branch has suggested that intraoperative infusions of large amounts of stored, bank blood may be an etiologic factor in producing postoperative pulmonary insufficiency. We have instituted clinical trials with blood filtration and studied the feasibility of intraoperative allotransfusion. Our studies have demonstrated a high degree of microaggregate formation in units of bank blood. As a result, the Pall Ultipore filter is now used on intraoperative blood administration sets. Intraoperative allotransfusion has been evaluated as an alternative to large amounts of bank blood. In intact animals, dead space, V_D/V_T , alveolar-arterial gradients, percent venous admixture, mean pulmonary arterial pressure, and pulmonary vascular resistance were significantly but transiently elevated. The effects of allotransfusion upon blood chemistry and the integrity of formed blood elements were examined in the human undergoing surgery. Evidence of severe hemolysis was found in allotransfused blood with markedly elevated levels of plasma free hemoglobin, SGOT, LDH and CPK; and diminished hematocrit, hemoglobin, and erythrocyte count. Microaggregation was found in both bank and allotransfused blood. Contrary to the acidotic pH of bank blood, the mean pH of allotransfused blood was 7.72. Further refinements in pump design are required before routine human use is feasible.

In cooperation with the Diagnostic Radiology and the Metabolic Disease Departments, work has continued on developing techniques for localizing parathyroid

adenomas by selectively catheterizing thyroid veins and radioimmunoassay of PTH. These techniques have proved very helpful clinically, particularly in the re-exploration for parathyroid adenomas.

Efforts have been made to improve cryoinstrumentation to facilitate the clinical application of these techniques. We have developed an omnidirectional probe clamp, which allows mobilization of the cryoprobe in an operating microscope stand that has been coupled with a recently developed ultraminiature multiple thermocouple to allow precision freezing. Experimental freezing of nerve tissue both in dogs and in primates has established that both the facial nerve and the recurrent nerve will return to normal full function after cryodestruction of surrounding tissues.

Using the I^{125} iododeoxyuridine (IDU) labelling technique developed by the Branch, host immunity to growing antigenic sarcomas was studied in a methylcholanthrene-induced guinea pig tumor system and in patients with skeletal and soft tissue sarcomas. Tumor-specific cellular immunity was assayed by cell-mediated cytotoxicity. Sera from animals and patients with growing sarcomas were evaluated for specific inhibition of this cellular immunity. The degree of tumor-specific cellular immunity in tumor-bearing animals and tumor-free animals was similar. However, sera from animals with palpable tumors inhibited specific cellular cytotoxicity. Sera drawn two weeks after tumor removal were non-inhibitory. These data suggest that inhibition is mediated by enhancing antibody. Similarly, human sarcoma-specific cellular cytotoxicity was present in most sarcoma-bearing patients studied. Sera from 14 out of 16 sarcoma-bearing patients inhibited sarcoma-specific cellular immunity, but did not inhibit cellular immunity against carcinoma. Serum inhibition correlated with the clinical stage of the disease. These data demonstrate an immunological phenomenon which may be related to the finding of progressive sarcoma growth in the presence of tumor-specific cell-mediated immunity.

A humoral assay was developed in which I^{125} IDU was used as a tumor cell label. This microassay was developed to detect cytotoxic tumor-specific antibody against I^{125} IDU labelled sarcoma cells in mice. The technique uses small amounts of serum and cells and is simple, sensitive, and objective. The cytotoxicity as determined by radioactive counting of cells using this technique correlates well with cytotoxicity as determined by visual counting.

A simplified isotopic antiglobulin assay was developed for the study of tumor-specific antibody. This test is similar to the indirect immunofluorescence test. Both test target cells against immune serum which is then tagged with a reagent that attaches to the immune serum. In the indirect immunofluorescence test, this reagent is a fluorescein-labelled antiglobulin, but in the isotopic antiglobulin test the antiglobulin is labelled with I^{125} . Previously, this test has been performed in suspension. The current modifications allow the test to be performed with plated tissue culture monolayers and using a simplified washing procedure, the result being a rapid and sensitive technique for detecting antibody against tumor-specific antigens.

A complement fixation assay demonstrated specific serum reaction with antigens in methylcholanthrene-induced sarcomas of guinea pigs. Sera from guinea pigs

given intradermal inoculations of viable sarcoma cells contained a complement fixing antibody that reacted specifically with antigens prepared from the immunizing tumor. The findings using the isotopic antiglobulin assay as well as the complement fixation assay indicate that the humoral immune response to these tumors should be considered in models of tumor immunotherapy.

The Branch has investigated cross-reactions between fetal antigens and tumor antigens in experimental animal tumors utilizing the guinea pig, rat, and mouse. Immunization of animals to second trimester (allogeneic or syngeneic) fetal tissue affords some degree of protection compared to control animals immunized to allogeneic adult tissue when challenged with various chemically-induced tumors. Although some resistance to tumor challenge has been shown in female exbreeder animals following multiple pregnancies, this has not been a consistent finding in all three animal systems. In addition, guinea pigs and rats were immunized to second trimester syngeneic fetal tissue or chemically-induced syngeneic tumors and subsequently skin tested with cell membrane or soluble antigen extracts of the tumors and found to show positive skin tests to the tumor antigen in both groups of animals, those immunized against the tumor and those immunized against the second trimester fetal tissue.

The effect of immunization of Balb/c mice with BCG vaccine on the subsequent development of melanoma following intramuscular challenge with Cloudman melanoma cells has been studied. Seven weeks following tumor challenge the overall tumor incidence in the BCG immune group was 34% lower than in the control group. The decreased tumor incidence in the BCG immune animals may have resulted from either nonspecific and new stimulation due to BCG immunization, or from specific rejection of melanoma cells due to sharing of common antigens by tubercle bacilli and melanoma. In vitro studies are underway to investigate these possibilities. Studies of preferential metastases have been carried out utilizing a spontaneously arising reticulum cell sarcoma in C₃H mice which selectively metastasizes to the spleen. Placement of these animals in parabiosis has confirmed the strong splenotropic tendency of these metastases. Study of this tumor in parabiotic animals provides a model which will permit evaluation of the role of immunologic factors in preferential tumor metastases. Studies of the effect of parabiosis on tumor growth rate carried out previously in the Branch have been confirmed and extended. Placement of a tumor-bearing animal in parabiosis in the normal animal results in inhibition of tumor growth rate. This is found not due to nonspecific factors such as surgery, confinement, or restraint. Recruitment of the immune response of the normal and immune animals joined in parabiosis with tumor-bearing animals may be responsible for the decreased tumor growth rate in the tumor-bearing animals. Studies to elucidate the mechanism of this effect are in progress.

Studies in guinea pigs utilized skin pedicles devoid of afferent lymphatic drainage as an immunologically privileged site to demonstrate that afferent lymphatics and regional lymph nodes are important for early host recognition of tumor allografts. However, regional lymph nodes were not found necessary for the rejection of tumor allograft, since systemic sensitization of the host eventually occurred. These findings suggest that once a tumor is sufficiently large to be detected clinically, the patient has already been sensitized systemically and would not benefit from leaving local lymph nodes intact,

especially since many nodes will contain metastatic tumor. Prevention of tumor growth in immunologically privileged sites was found possible by adoptive transfer of cell-mediated tumor-specific immunity using immune spleen cells administered intraperitoneally.

TABLE 1
Individual Written Intramural Consultations Received by Surgery Branch, NCI
Listed by Institutes
1963-1972

	<u>NCI</u>	<u>NINDS</u>	<u>NIAMD</u>	<u>NHLI</u>	<u>NIAID</u>	<u>NIMH</u>	<u>NICHD</u>	<u>NIDR</u>	<u>NEI</u>	<u>EHS</u>	<u>TOTAL</u>
1963	366	184	221	228	158	37	-	26	-	-	1220
1964	356	121	207	201	104	33	-	24	-	-	1046
1965	429	129	216	277	119	39	-	31	-	-	1240
1966	362	271	201	197	86	59	-	11	-	7	1205
1967	343	215	245	166	85	61	-	17	-	-	1132
1968	278	143	192	203	111	54	-	25	-	7	1013
1969	314	131	248	207	128	45	-	31	-	10	1114
1970	199	59	137	104	96	41	-	12	-	7	655
1971	220	98	152	89	94	62	15	28	28	18	804
1972	189	72	134	71	98	58	8	15	10	14	669

TABLE 2
Summary of Written Extramural Consultations Requested by or Through
Surgery Branch, NCI
1963-1972

	<u>ENT</u>	<u>General Surgery</u>	<u>Gynecology</u>	<u>Urology</u>	<u>Oncology</u>	<u>Total</u>
1963	346	14	7	18	9	394
1964	233	6	4	4	3	242
1965	268	6	2	9	1	286
1966	271	8	4	13	2	298
1967	294	11	1	1	1	308
1968	306	8	1	1	1	317
1969	329	8	2	3	2	344
1970	778	12	2	2	0	794
1971	704	3	0	0	0	707
1972	1034	20	0	0	0	1054

TABLE 3

Annual Report of NIH Surgical Suites
 Listing the Number of Major & Minor Procedures
 Carried Out by Each of the Operative Units at NIH
 April 1, 1972 - March 31, 1973

Patient	NCI	NHLI	NINDS Neuro. Muscle	NIAID	NIAMD	NEI	NIMH	NICHD	NIDR	CONSULTANTS	Total #Pt. Operated
NCI Major Minor	247 329	7 5	12 -	- 4	- 1	- 1	- -	1 1	- 2	21 16	290 649 359
NHLI Major Minor	20 20	202 24	- 1	- 3	- -	1 1	- -	1 1	- 1	1 6	225 57 282
NINDS Major Minor	3 5	1 1	80 19	- 178	- -	- -	- -	- -	- 1	1 4	85 208 293
NIAMD Major Minor	29 13	2 -	- 1	- 21	- -	2 -	- -	- -	- -	11 11	44 46 90
NIAID Major Minor	7 26	3 2	- -	- 6	- -	3 1	- -	- -	- 1	- 21	13 57 70
NIDR Major Minor	- 2	1 -	- -	- -	- -	- -	- -	- -	- 3	1 -	2 5 7
NIMH Major Minor	- 5	- -	- -	- -	- -	- -	- -	- -	- -	- 1	0 6 6
NICHD Major Minor	2 12	- -	2 5	- -	- -	- -	- -	4 6	- 1	4 1	12 25 37
NEI Major Minor	1 3	- -	- -	- -	- -	36 12	- -	- -	- -	- -	37 15 52

TABLE 3 (CONTINUED)

MAJOR TOTAL	309	216	94	0	0	0	44	0	6	0	39	708
MINOR TOTAL	<u>415</u>	<u>32</u>	<u>26</u>	<u>212</u>	<u>1</u>	<u>0</u>	<u>15</u>	<u>0</u>	<u>8</u>	<u>9</u>	<u>60</u>	<u>778</u>
GRAND TOTAL	724	248	120	212	1	0	59	0	14	9	99	1486*

*Of these 1486 cases, 94 patients were on OPD status.

Cardiac Cath. 7N-126
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2049

TABLE 4

Number of Surgical Procedures Performed
by NCI Surgery Branch Staff
and Magnitude of the Procedures in 1972
April 1, 1972 - March 31, 1973

	<u>10 East</u>	<u>Other NCI</u>	<u>Other Institutes</u>	<u>Total</u>
1963	470	159	399	1028
1964	424	215	256	895
1965	394	112	199	705
1966	410	113	218	741
1967	440	145	140	725
1968	394	205	113	712
1969	339	233	158	730
1970	301	273	230	804
1971	228	273	179	680
1972	307	146	136	589
Major	183	88	64	335
Minor	124	58	72	254

Serial No. NCI-3800
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Removal of Cancer and Other Tissues for Histologic, Biochemical and Other Studies as Required by Scientists and Other Investigators for Correlated Studies

Previous Serial Number: Same

Principal Investigators: Entire Staff of the Surgery Branch, NCI

Other Investigators: G.D. Aurbach, M.D., P.P. Carbone, M.D., V.T. DeVita, M.D., R.I. Henkin, M.D., Ph.D., R.E. Johnson, M.D., F.C. Bartter, M.D., L.B. Thomas, M.D., J. Linsk, M.D., E.W. Chu, M.D., J.L. Ziegler, M.D., and J.L. Doppman, M.D.

Cooperating Units: Metabolic Disease Branch, NIAMD; Medical Oncology, NCI; Medicine Branch, NCI; Experimental Therapeutics Branch, NHLI; Radiation Branch, NCI; Endocrinology Branch, NHLI; Laboratory of Pathology, NCI; Cytopathology Department, Atlantic City Hospital, Atlantic City, New Jersey; Cytopathology Section, Lab. of Pathology, NCI; Pediatric Oncology Branch, NCI; and Department of Diagnostic Radiology, Clinical Center

Man Years:

Total: 6-1/2
Professional: 4
Other: 2-1/2

Project Description:

Part I. Service Aspects

Part II. Specific Cooperative Studies

- A. Pheochromocytoma
- B. Hyperparathyroidism
- C. Thyroid Carcinoma
- D. Lymphoma and Hodgkin's Disease
- E. Splenectomy and its Role in Chronic Myelogenous Leukemia
- F. Treatment of Breast Cancer

Project Description:Part I. Service AspectsObjectives:

The object of this project is to make available human tissue for histologic, histochemical, biochemical, biophysical, viral, and immunological studies to scientists working in various Branches of the National Cancer Institute and other Institutes who require tissue for studies. As a rule, such studies are not done as cooperative projects. In addition, it implies furnishing general surgical consultative coverage of patients admitted to other Branches of the National Cancer Institute and/or other Institutes and assisting with their study projects. This surgery is often definitive in nature, the therapy regime requiring surgical tissue excision as the sole type of therapy, or more often as an adjuvant to other modes of treatment.

Methods Employed:

Methods employed are standard surgical procedures. Material from this project is obtained mostly from patients hospitalized for study in other Branches or Institutes. Occasionally it is necessary to admit patients for specific studies in which a specific type of tissue is obtained. Tumor tissue, lymph nodes, muscle, skin, and liver and spleen comprise the greater share of tissue removed.

Major Findings:

In that the major portion of the work involved in this project is done as a surgical consultative service and not as a cooperative study, the project results are not immediately available to the surgical service. During the course of supplying this service, 669 individual requests for consultation were received. These figures do not include the extensive follow-up which is afforded each of these consultations, but only the original written request to participate in their care. Of these requests, 282 resulted in an operative procedure, 35% of which entailed major surgery. See attached Tables 1-4.

Significance to Biomedical Research and Program of the Institute:

This project is a service function for other Branches of the National Cancer Institute and National Institutes of Health. It is seldom that the Surgery Branch is included in the research studies other than supplying the tissue to the investigator; however, this trend is being reversed and several studies are being carried out on a cooperative basis with Surgery Branch Staff now serving as active counselors in the overall study.

Proposed Course:

Continued cooperative assistance within the physical limits of the Surgery Branch Staff.

Project Description:Part II. Specific Cooperative StudiesA. PheochromocytomaObjectives:

The goals of this project have been: 1) the development and evaluation of a joint medicosurgical program for the overall management of patients with pheochromocytoma and 2) continuing clinicopathologic scrutiny of the operative material from these cases in an effort to define more clearly the malignant potential possessed by this unusual tumor which lacks the usual histologic criteria for malignancy in spite of demonstrating its biologically malignant nature in about 10% of cases.

Methods Employed:

Standard surgical and anesthetic procedures are used when operating upon these patients. The analytical methods for catecholamine determinations are those developed and currently employed by the Experimental Therapeutics Branch, NHLI. The patients studied are those who have been referred to the Experimental Therapeutics Branch, NHLI, for evaluation and treatment of their pheochromocytoma. Both conventional light and electron microscopy are applied to the study of the resected materials. Attempts are underway to grow these tumors in tissue culture in order to develop a means for assessing biologic malignant potential by an in vitro procedure.

Major Findings:

Our experience includes 33 cases. Intraoperative localization of tumor by observation of blood pressure rise in response to manipulation remains satisfactory. Thorough preoperative biochemical preparation of these patients continues to provide a much safer anesthetic and operative course. More data has accumulated to strengthen our concept that total adrenalectomy should be done in all instances of adrenal pheochromocytoma rather than attempt to shell tumor out of a gland which is to be left behind. Preliminary results with tissue culture of pheochromocytoma material suggest that a clinical and histologically benign pheochromocytoma currently cannot be developed into a serially transferable tissue culture line. Further experience with a more malignant lesion is necessary.

Significance to Biomedical Research and Program of the Institute:

There are a few tumors which have a dual uniqueness similar to pheochromocytoma, i.e., a functional lesion with an easily measured secretory produce, but which lacks intrinsic histologic criteria of malignancy. This combination provides a rare opportunity to study the effectiveness of vigorous surgical therapy using as an endpoint something other than the ultimate demise of the patient from disseminated disease.

The demonstration that these tumors will grow for a time in tissue culture, although their transferability into a permanent line has not been achieved as yet, suggests that this means may be useful in assaying degree of malignant potential prior to the development of lethal biological evidence in the patient. The measurement of catecholamines in the tissue culture supernatant and in the cells themselves offers further potential of evaluating efficacy of agents that might have specific antitumor activity.

Proposed Course:

A review that is underway will have emphasis placed upon elucidation of relative degrees of malignancy among the various lesions and upon attempts to benefit more patients with malignant pheochromacytomas by meticulous surgery.

Project Description:

Part II. Specific Cooperative Studies

B. Hyperparathyroidism

Objectives:

The Clinical Endocrinology Branch, NHLI, the Section on Mineral Metabolism, Metabolic Diseases Branch, NIAMD, and the Diagnostic Radiology Branch, Clinical Center, have programs underway to determine clinical, laboratory, and radiographic criteria which justify surgical exploration of the neck for the treatment of parathyroid disease. This joint program is expected to increase the reliability of diagnosis and efficacy of treatment for parathyroid disorders, particularly in early cases where the treatment will reduce the incidence of secondary renal disease.

Methods Employed:

Standard surgical and anesthetic procedures are used for operating on the patients. In addition to the customary extensive clinical evaluation and laboratory determinations of static derangements of calcium and phosphorus metabolism, a battery of tests are performed to measure responses to induced distortions of calcium and phosphorus hemostasis. Additionally, selective inferior thyroid arteriographic studies are done preoperatively, as is selective venography and removal of blood samples from various veins draining the parathyroid complex identified by venography. Radioimmunoassay determinations of parathyroid hormone are made upon the venous samples so obtained. At operation, prior to any extirpative procedure, intensive efforts are made to identify positively by frozen section all parathyroids present.

Major Findings:

Selective inferior thyroid arteriography continues to be of considerable value in the preoperative localization of abnormal parathyroid glands, particularly when the subtraction technique is applied to the arteriograms. Selective venography has not been helpful in finding abnormal parathyroid glands, but has

enabled the radiographer to identify the appropriate veins from which to withdraw blood for parathyroid hormone assays. The parathyroid hormone assays have been extremely encouraging in that out of 31 patients studied the lateralization of the parathyroid neoplasm has been successfully predicted in all 30 cases. This technique is extremely helpful in patients in which adenoma was not found at the first surgery.

Significance to Biomedical Research and Program of the Institute:

Early diagnosis and removal of overactive parathyroid tissue is an important step toward preservation of renal function from the ravages of stone formation. The addition of selective venous catheterization and radioimmunoassay for parathyroid hormonal levels of samples thereby obtained is an important tool for both diagnosis of primary hyperparathyroidism and preoperative localization of the abnormal tissue. Selected angiography has continued to bear out its earlier promise as an important aid to the surgeon in finding the abnormal glands readily, with a resultant decrease in operating time and morbidity.

Proposed Course:

Continued cooperative studies.

Project Description:

Part II. Specific Cooperative Studies

C. Thyroid Carcinoma

Objectives:

To assess the clinical, pathological, and endocrinological parameters of patients with Sipple's syndrome (medullary carcinoma of the thyroid gland, pheochromocytoma, and parathyroid hyperplasia).

Methods Employed:

Presently a large number of patients have been admitted to the NIH with the diagnosis of Sipple's syndrome. These patients have been diagnosed positively by the presence of an elevated thyrocalcitonin level in their peripheral blood. Patients with peripheral levels above 0.3 nanograms/ml. can be diagnosed with 100% accuracy as having medullary carcinoma of the thyroid gland. A large number of these patients have concurrent pheochromocytomas and parathyroid hyperplasia. Large numbers of this family have been screened and admitted to the NIH for study. The disease status is diagnosed and they subsequently undergo operation for removal of the thyroid carcinoma and other disease organs should they be manifest.

Major Findings:

The elevated peripheral level of thyrocalcitonin has been an excellent screening

test for the presence of medullary carcinoma of the thyroid gland. We have had no false positive findings in that all patients with levels above 0.3 nanograms/ml. have thyroid carcinoma. At present, of all of the serological tests for the detection of cancer, this one has the greatest accuracy. It has also been found that histaminase levels are elevated once the disease metastasizes outside of the thyroid gland. This is a good index of measuring extent of disease in those patients who either have undergone surgery or in those who have recurred or who have residual tumor left behind following surgery. The standard operative procedure that has been employed in these patients has been a total thyroidectomy with removal of the central nodes of the neck. Most of the patients in our group have had generalized parathyroid hyperplasia and have undergone subtotal parathyroidectomy.

Significance to Biomedical Research and Program of the Institute:

This methodology has been extremely important in that the serological screening test has had a high degree of accuracy in the thyroid carcinoma. This allows the early diagnosis of medullary carcinoma of the thyroid gland and hopefully total removal of the neoplasm before metastasis has occurred.

Proposed Course:

To screen the remainder of the pedigree and admit those diseased for study and surgery.

Project Description:

Part II. Specific Cooperative Studies

D. Lymphoma and Hodgkin's Disease

Objectives:

To assess the adequacy of staging of patients with lymphoma and Hodgkin's disease by nonsurgical methods. To assess the effect of splenectomy on patient survival and tolerance of radiotherapy and chemotherapy treatment regimens.

Methods Employed:

All patients admitted to the Chemotherapy and Radiotherapy Services with lymphoma and Hodgkin's disease undergo routine staging by the combined evaluation of clinical and physical findings, liver chemistries, lymphangiograms, percutaneous liver biopsy, peritoneoscopy, liver biopsy, and chest x-ray. To assess the accuracy of these staging techniques, selected patients have been subjected to laparotomy and biopsy of the liver and abdominal lymph nodes. Selected adenopathy detected after treatment is further evaluated by biopsy and histologic examination.

Major Findings:

The mounting experience gained through the examination of tissues removed at laparotomy in patients with lymphoma and Hodgkin's disease in comparison with

staging data will more accurately delineate those patients who will profit from the inconvenience of laparotomy. Sufficient evidence has accumulated to allow the conclusion that all patients will not benefit from staging laparotomy, a conclusion that is at variance with the findings of several other major treatment centers. On the other hand, biopsy of multiple abdominal nodal areas has demonstrated the limitations of lymphangiography in assessing the extent of abdominal nodal disease. The contribution made by abdominal laparotomy and biopsy, however, is clearly dependent on the treatment proposed and how it is altered by the finding of unsuspected positive abdominal lymph nodes.

A sequential evaluation of the liver by percutaneous biopsy, followed by peritoneoscopy-biopsy in patients with negative percutaneous biopsies, shows that the second biopsy averts open biopsy in a significant incidence of patients. For instance, in non-Hodgkin's lymphomas, only 10 of 24 consecutive patients required open biopsy, because in 10 patients positive tissue was obtained by percutaneous biopsy, and in 4 additional patients biopsy with peritoneoscopic control yielded positive tissue.

The evidence emerging from other treatment centers that splenectomy favorably affects patient survival and tolerance of radiotherapy and chemotherapy in Hodgkin's disease and lymphosarcoma is currently being assessed at this institution by a prospective study of the effects of splenectomy on these parameters. Although the value of splenectomy in those patients with abnormal consumption of blood cells is continuously being demonstrated as is the laboratory benefit of splenectomy and the salutary benefit of removal of spleens unresponsive to chemotherapy and radiotherapy, sufficient data has not yet accumulated to allow assessment of the effects of splenectomy on tolerance of subsequent specific treatment and overall survival.

Significance to Biomedical Research and Program of the Institute:

The cooperation of the Surgery Branch with the divisions of the Institute concerned with treatment of patients with lymphoma and Hodgkin's disease results in a continuously improving program of diagnosis and treatment of these patients. These studies allow the intelligent selection of those patients who would benefit by laparotomy prior to specific treatment of the disorder.

Proposed Course:

Studies in progress will correlate laparotomy findings in these diseases with response to therapy and long-term prognosis. The effect of splenectomy on tolerance to chemotherapy and radiotherapy will be determined.

Project Description:

Part II. Specific Cooperative Studies

E. Splenectomy and Its Role in Chronic Myelogenous Leukemia

Objectives:

To reassess the effect of splenectomy on normalization of blood cell kinetics in patients with chronic myelogenous leukemia (CML); to delineate those patients that will benefit from splenectomy.

Methods Employed:

Thrombocytopenia is usually a late manifestation of CML. Most often it is associated with transformation of the disease to the blastic phase. In a smaller number of patients thrombocytopenia, which may be a direct manifestation of the disease process or result from an inordinate sensitivity to the myelosuppressive effects of chemotherapy, occurs in the chronic stage. This latter instance severely limits the effective treatment of the disease and may require hospitalization and platelet transfusion support. In patients presenting with this clinical picture splenectomy is performed. The effects of this operative procedure on the subsequent hematologic picture and tolerance to therapy is evaluated.

Major Findings:

The indications for splenectomy in this group of patients fell into two general categories: (1) The inability to control the clinical and hematological manifestations of the disease because of an inordinate sensitivity of platelet blood counts to busulphan; and (2) prolonged pancytopenia and severe thrombocytopenia secondary to busulphan myelosuppression, resulting in frequent hospitalization for multiple platelet transfusions. All patients tolerated the operation without excessive bleeding. Platelet concentrate transfusions were utilized throughout the procedure and during the postoperative period. There was an almost immediate rise in the platelet count within 48 hours in all patients. The extent of postoperative thrombocytosis did not appear to correlate with spleen size at the time of surgery. In one case, the spleen histology predicted blastic transformation, showing extensive replacement of the spleen by immature blast cells. In the other cases, the histopathology was compatible with CML or myeloid metaplasia. There appeared to be no correlation between the numbers of megakaryocytes in the marrow and subsequent response to therapy. The postoperative rise in platelet count was rapid in most patients. In all patients with hemorrhagic manifestations of thrombocytopenia, splenectomy ameliorated the bleeding and obviated the need for further platelet transfusion.

Significance to Biomedical Research and Program of the Institute:

This study delineates a role for splenectomy for the treatment of CML. Those patients with prolonged thrombocytopenia and hemorrhagic manifestations due to busulphan-related myelosuppression unresponsive to corticosteroids and frequent platelet transfusions and those with inordinate platelet sensitivity to chemotherapy are candidates for splenectomy. The rapid rise in platelet count and cessation of hemorrhagic complications were gratifying results in these patients. This information is of critical importance to all involved in the exploration of improved therapeutic approaches to the treatment of this disease.

Proposed Course:

This project will continue, with further exploration of the beneficial effect of splenectomy on the course of patients with CML. These include exploration of less obvious effects, including immunologic alterations, precipitated by splenectomy.

Project DescriptionPart II. Specific Cooperative StudiesF. Treatment of Breast CancerObjectives:

To develop an expanded program for the treatment of breast cancer through its diagnosis, primary surgical treatment, first recurrence (pre- and post-pausal), adjunctive chemotherapy, and metastatic disease by means of a multidisciplinary approach.

Methods Employed:

Breast cancer is a leading killer of women in the prime of life in the United States. As the number one cancer in the majority of the population, a concerted effort is being developed in the National Cancer Institute by combining forces against this leading cause of cancer morbidity and mortality in the United States. The Breast Oncology Section of the Surgery Branch of NCI has joined forces with a medical breast unit counterpart in the Breast Tumor Unit which has been developed and is growing rapidly this year. Combined protocols have been drawn up which utilize the services of every discipline that can be of therapeutic benefit to the breast cancer patient. Extensive literature review and careful planning have gone into the kind of breast program that would incorporate the latest advances in cancer control in this Breast Cancer Service which has only recently begun admitting patients. The Surgery Branch is intimately involved in the evolution of the Breast Cancer Service and has been and will be a major input in the design, planning, work-up, referral, decision, and care of these cancer patients. The fundamental programs that the Surgery Branch has participated in developing have been in the design of the protocols for patient referral, questionnaires for epidemiologic information, physical examination forms, staging and pathology records, radiotherapy records, laboratory investigations, and all of the records and data gathering plans formulated in the "BTS 1-28" with their associated charts and flow diagrams. The specific protocols which have been designed for the help of the Surgery Branch input and which will require the Surgery Branch's services and diagnostic work-up, staging, biopsy, definitive primary surgical care, palliative resection, endocrine ablative procedures, and therapeutic decisions are as follows:

1. BTS 131: Study to Evaluate the Effective Diagnostic Procedures in Examinations and Early Detection of Mammary Carcinoma in Populations at High Risk.

The Surgery Branch Breast Oncology Section is the prime mover in this screening for treatment into ongoing therapy programs within the Breast Tumor Section. Evaluation of techniques of diagnosis such as tumor marker assays, urinary Bulbrook Discriminant, physical examination, history, mammography, thermography, zerography, fine needle aspiration, vacuum aspiration, radionuclide studies, and biopsy will be performed with a major emphasis on patient education and self-examination. Cytopathologic protocols also depend on the screening protocol for the aspiration cytology, examination of routine vaginal smears for hormonal levels, sex chromatin bodies, buccal mucosal cells, chromosomal analysis, cytochemical studies on tumor cells, and electron microscopic studies of cell surfaces. The study will seek to identify a population at high risk for the development of breast cancer and follow them with a combination of the best diagnostic modalities in order to be able to detect a developing breast cancer at its earliest possible stage for the best possible treatment results.

2. BTS 141: Primary Surgical Therapy for Breast Cancer

The Surgery Branch will undertake primary surgical treatment of the breast cancers referred to it and discovered by the screening clinic. The operation to be done at this time will be a classic Halsted radical mastectomy or a modified radical mastectomy so as to remove the whole of the breast substance with the contents of the axilla. At a later date, some consideration might be made to having a randomized protocol of lesser surgical procedures, but at this present time, for the therapeutic benefit of this standard operative approach and for the information obtained from the staging by axillary node examination, radical or modified radical mastectomy are to be done on all operable breast cancers in the Surgery Branch

A protocol is under consideration in the Surgery Branch whereby anticoagulation therapy would be adjunctive to mastectomy in the primary surgical treatment of breast cancer. In this study, patients would be randomized to being treated by surgery alone or surgery with pre- and postoperative anticoagulation therapy as has been in practice on the Coumadin protocol for sarcoma. This protocol is in the process of development and has not as yet been approved for institution

3. Adjuvant Disease Protocol

Cooperative Participation in National Trials

- A. National Surgical Adjuvant Breast Project Using Phenylalanine Mustard Versus Placebo.
- B. Acute Leukemia Group B Study 728 Studying Phenylalanine Mustard Versus Phenylalanine Mustard, 5FU, and Methotrexate.
4. BTS 125: NIH Protocol Randomizing Phenylalanine Mustard Versus CCNU, Phenylalanine Mustard, Methotrexate, and 5FU.

These adjuvant programs will be used in patients at high risk for recurrence following surgical therapy. These will be identified as those patients having four or more nodes positive in their radical mastectomy specimen.

5. BTS 123: Study to Evaluate the Effect of Adjuvant Chemotherapy in the Management of Recurrent Mammary Carcinoma in Pre-Menopausal Women.

Patients entering this protocol will have an oophorectomy and the responders will be randomized to cyclophosphamide, adriamycin, 5FU, and prednisone maintenance to no therapy. Those patients that relapse will crossover to treatment with the drugs outlined above to adrenalectomy. Both oophorectomy and adrenalectomy are surgical services of the Breast Oncology Section of the Surgery Branch as well as decisions and evaluations on the patients during their course.

6. Protocol B124: Evaluation of Adjuvant Chemotherapy in Recurrent Breast Cancer in Post-Menopausal Women

This study will be randomized similar to the one outlined above without the prior oophorectomy and with the later addition of estrogens as outlined in the protocols.

7. BTS 132: Evaluation of Adjuvant Chemotherapy in Breast Carcinoma Patients with no Evidence of Disease Following Excised Mastectomy Site Recurrence.

These patients who qualify for this protocol will undergo laparotomy and oophorectomy and randomized to no further therapy versus cyclophosphamide, methotrexate, 5FU.

8. BTS 122: Metastatic Breast Cancer Carcinoma Study to Evaluate the Effect of Cyclophosphamide, Methotrexate, 5-Fluorouracil versus Cyclophosphamide, Adriamycin, and 5-Fluorouracil.

This study will evaluate eligible patients who have not received previous chemotherapy, but who present with, or are referred with, metastatic breast cancer.

Significance to Biomedical Research and Program of the Institute:

Treatment of breast cancer is a rapidly expanding interest within the Surgery Branch of NCI. In combining efforts in a collaborative Breast Tumor Section, all modalities of therapy are being directed against this most significant cancer in this society. Patients treated within the Surgery Branch will be followed closely by surgeons' medical counterparts for easy access to chemotherapy for recurrent disease both for the patient's benefit and for accrual of information to augment the protocols set up within NCI.

Proposed Course:

The expansion and further development of the protocols presently written and being instituted is planned, and expansion of the newly organized Breast Tumor Section is planned for the coming year.

TABLE 1
Individual Written Intramural Consultations Received by Surgery Branch, NCI
Listed by Institutes
1963-1972

	<u>NCI</u>	<u>NINDS</u>	<u>NIAMD</u>	<u>NHLI</u>	<u>NIAID</u>	<u>NIMH</u>	<u>NICHD</u>	<u>NIDR</u>	<u>NEI</u>	<u>EHS</u>	<u>TOTAL</u>
1963	366	184	221	228	158	37	-	26	-	-	1220
1964	356	121	207	201	104	33	=	24	=	=	1046
1965	429	129	216	277	119	39	-	31	-	-	1240
1966	362	271	201	197	86	59	-	11	-	7	1205
1967	343	215	245	166	85	61	-	17	-	-	1132
1968	278	143	192	203	111	54	-	25	-	7	1013
1969	314	131	248	207	128	45	-	31	-	10	1114
1970	199	59	137	104	96	41	-	12	-	7	655
1971	220	98	152	89	94	62	15	28	28	18	804
1972	189	72	134	71	98	58	8	15	10	14	669

TABLE 2
Summary of Written Extramural Consultations Requested by or Through
Surgery Branch, NCI
1963-1972

	<u>ENT</u>	<u>General Surgery</u>	<u>Gynecology</u>	<u>Urology</u>	<u>Oncology</u>	<u>TOTAL</u>
1963	346	14	7	18	9	394
1964	233	6	4	4	3	242
1965	268	6	2	9	1	286
1966	271	8	4	13	2	298
1967	294	11	1	1	1	308
1968	306	8	1	1	1	317
1969	329	8	2	3	2	344
1970	778	12	2	2	0	794
1971	704	3	0	0	0	707
1972	1034	20	0	0	0	1054

TABLE 4

Number of Surgical Procedures Performed by NCI Surgery Branch Staff
and Magnitude of the Procedures in 1972

April 1, 1972 - March 31, 1973

	<u>10 East</u>	<u>Other NCI</u>	<u>Other Institutes</u>	<u>Total</u>
1963	470	159	399	1028
1964	424	215	256	895
1965	394	112	199	705
1966	410	113	218	741
1967	440	145	140	725
1968	394	205	113	712
1969	339	233	158	730
1970	301	273	230	804
1971	228	273	179	680
1972	307	146	136	589
Major	183	88	64	335
Minor	124	58	72	254

TABLE 3
Annual Report of NIH Surgical Suites
Listing the Number of Major & Minor Procedures
Carried Out by Each of the Operative Units at NIH
April 1, 1972 - March 31, 1973

Patient	NCI	NHLI	NINDS Neuro. Muscle	NIAID	NIAMD	NEI	NIMH	NICHD	NIDR	CONSULTANTS	Total #Pt. Operated
NCI Major Minor	247 329	7 5	12 -	- 4	- 1	2 1	- -	1 1	- 2	21 16	290 359
NHLI Major Minor	20 20	202 24	- 1	- 3	- -	1 1	- -	1 1	- 1	1 6	225 57
NINDS Major Minor	3 5	1 1	80 19	- 178	- -	- -	- -	- -	1 1	1 4	85 208
NIAMD Major Minor	29 13	2 -	- 1	- 21	- -	2 -	- -	- -	- -	11 11	44 46
NIAID Major Minor	7 26	3 2	- -	- -	- -	3 3	- -	- -	- -	- -	13 57
NIDR Major Minor	- 2	1 -	- -	- -	- -	- -	- -	- -	- 3	1 -	2 5
NIMH Major Minor	- 5	- -	- -	- -	- -	- -	- -	- -	- -	- 1	0 6
NICHD Major Minor	2 12	- -	2 5	- -	- -	- -	- -	4 6	- 1	4 1	12 25
NEI Major Minor	1 3	- -	- -	- -	- -	36 12	- -	- -	- -	- -	37 15
MAJOR TOTAL	309	216	94	0	0	44	0	6	0	39	708
MINOR TOTAL	415	32	26	1	0	15	0	8	9	60	778
GRAND TOTAL	724	248	120	212	1	59	0	14	9	99	1486*

* Of these 1486 cases, 94 patients were on OPD status.

Cardiac Cath. 7N-126

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Publications:

Shimkin, P.M., Doppman, J.L., Powell, D., Marx, S.J., and Ketcham, A.S.: Demonstration of parathyroid adenomas by retrograde thyroid venography. Radiology 103:63-67, 1972.

Powell, D.P., Shimkin, P.M., Doppman, J.L., Wells, S.A., Aurbach, G.D., Marx, S.J., Ketcham, A.S., and Potts, J.T., Jr.: Primary hyperparathyroidism: Pre-operative tumor localization and differentiation between adenoma and hyperplasia. New Eng. J. Med. 286:1169-1175, 1972.

Shimkin, P.M., Powell, D., Doppman, J.L., Marx, S.J., Pearson, K.D., Wells, S.A., and Ketcham, A.S.: Parathyroid venous sampling. Radiology 104:571-574, 1972.

Wells, S.A., Ketcham, A.S., Marx, S.J., Powell, D., Bilezikian, J.P., Shimkin, P., Potts, J.T., Jr., Pearson, K.D., and Doppman, J.L.: Preoperative localization of hyperfunctioning parathyroid tissue: Radioimmunoassay of parathyroid hormone in plasma from selectively catheterized thyroid veins. Ann. Surg. 177: 93-98, 1973.

Bilezikian, J.P., Aurbach, G.D., Connor, T.B., Pachas, W.N., Aptekar, R., Wells, S.A., Jr., Freijanes, J., and Decker, J.L.: Pseudogout after parathyroidectomy. Lancet 1:445, 1973.

Geelhoed, G.W., Graff, K.S., Duttera, M.J., and Henderson, E.S.: Acute leukemia presenting as a breast mass. J.A.M.A. 223:1488-1489, 1973.

Marx, S.J., Powell, D., Shimkin, S., Wells, S.A., Ketcham, A.S., McGuigan, J., Bilezikian, J., and Aurbach, G.D.: Familial hyperparathyroidism. Ann. Intern. Med. 78:371-377, 1973.

Keiser, H.R., Beaven, M.A., Doppman, J.L., Wells, S.A., and Buja, L.M.: A large family with Sipple's syndrome: Medullary thyroid carcinoma, pheochromocytoma, and hyperparathyroidism. Ann. Intern. Med. 78:561-580, 1973.

Bagley, E.M., Thomas, L.B., Johnson, R.E., Chretien, P.B., and DeVita, V.T.: Diagnosis of liver involvement by lymphoma: Results in 96 consecutive peritoneoscopies. Cancer 31:840-847, 1973.

Bilezikian, J.P., Pearson, K.D., Powell, D., Ketcham, A.S., Doppman, J.L., and Aurbach, G.D.: Selective venous catheterization of the neck: Localization of a parathyroid adenoma drained anomalously by non-thyroidal veins. J. Clin. Endocrin. Metabol., in press.

Doppman, J.L., Wells, S.A., Shimkin, P.M., Pearson, K.D., Bilezikian, J.P., Powell, D., Ketcham, A.S., and Aurbach, G.D.: Parathyroid localization by angiographic techniques in patients with previous neck surgery. Brit. J. Radiol., in press.

Schein, P., Kahn, R., Gorden, P., Wells, S.A., and DeVita, V.T.: Streptozotocin treatment of malignant insulinomas and carcinoids: Report of eight cases and review of the literature. Arch. Intern. Med., in press.

Marx, S.J., Powell, S., Shimkin, S., Wells, S.A., Ketcham, A.S., McGuigan, J., Bilezikian, J., and Aurbach, G.D.: Familial hyperparathyroidism. Ann. Intern. Med., in press.

Johnson, R.E., Chretien, P.B., and O'Connor, G.T.: Influence of extensive biopsy sampling at laparotomy on radiotherapy planning in the non-Hodgkin's lymphomas. Proceedings of the 1972 Congress of German Radiologists, in press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3800

Title: Removal of Cancer and Other Tissues for Histologic, Biochemical, and Other Studies as Required by Scientists and Other Investigators for Correlated Studies

Principal Investigator: Entire Staff of the Surgery Branch, NCI

Section: Office of the Chief

Lab/Branch: Surgery Branch

Goal: To assist non-surgical units of NIH in the evaluation and treatment of patients admitted to their research protocols.

Approach: Patients receive comprehensive evaluations as determined by research protocols of the individual units of NIH. The Surgery Branch, NCI, assists in the surgical aspects of these evaluations and participates in the selection of patients that require definitive surgical treatment of the disease entities. Correlations are made between operative findings and results of preoperative diagnostic tests to better define the role of surgery in the diagnosis and treatment of the diseases studied.

Progress: Prerequisites for safe and expeditious surgery for pheochromocytoma have been defined. Preoperative localization of parathyroid adenomas has been perfected. The value of thyrocalcitonin levels in the evaluation of medullary thyroid carcinoma has been demonstrated. The role of surgical staging in Hodgkin's disease and lymphomas is being determined, along with the effect of splenectomy in these diseases and also in chronic myelogenous leukemia. Cooperative protocols for the treatment of breast cancer have been developed.

Significance for Cancer Research (NCP Objective 5, Approach 4): These studies determine the presence and extent of a variety of human neoplasms and utilize surgery for the evaluation of tests designed to improve methods of diagnosis and localization of tumors.

Level of Effort: Professional Man-Years: 4

1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Evaluation of Cancer Surgery as to its Effectiveness and Safety in Providing Definitive and Therapeutic Care of Cancer Patients

Previous Serial Number: Same

Principal Investigators: A.S. Ketcham, M.D., P.B. Chretien, M.D., and the entire Staff of the Surgery Branch, NCI

Other Investigators: L.B. Thomas, M.D., S. Kakehashi, M.D., R.E. Johnson, M.D., G.S. Johnston, M.D., J.L. Doppman, M.D., J.L. Ziegler, M.D., V.D. DeVita, M.D., W.T. Terry, M.D., J. Block, M.D., and M.J. Lotz, M.D.

Cooperating Units: Laboratory of Pathology, NCI; Dental Service Branch, NIDR; Experimental Therapeutics Branch, NHLI; Radiation Branch, NCI; Diagnostic Radiology Department; Department of Nuclear Medicine; Clinical Pathology Department; Nursing Department; Anesthesiology Department; Social Work Department; Rehabilitation Department; Clinical Center; NINDS.

Man Years:

Total:	8-1/2
Professional:	5
Other:	3-1/2

Project Description:

Part I. Study of the Biologic Behavior and Results of Surgical Therapy of Cancer of the Uterine Cervix

A. Surgical Treatment of Carcinoma of the Cervix

B. Scalene Nodes as Indicators of Disseminated Cancer

Part II. The Surgical Treatment of Melanoma

A. Surgical Staging of Melanoma Patients

Part III. Malignant Melanoma: Screening of Potential Surgical Candidates with ⁶⁷Gallium Scintigraphy

- Part IV. Fine Needle Aspiration Biopsy in the Diagnosis of Solid Tumors
- Part V. Medullary Extension of Osteosarcoma
- Part VI. Evaluation of Uretero-ileostomy Following Urinary Diversion
- Part VII. Axillary Dissection with Preservation of the Pectoralis Major Muscle
- Part VIII. Extended Deltopectoral Flap Reconstruction of Anterior Oral Defects
- Part IX. A Study of the Results of Surgical Treatment and Effects of Adjunctive Therapy in Cancer of the Head and Neck
 - A. A Retrospective Computer Analysis of Patients with Head and Neck Tumors Treated Surgically at the NIH
- Part X. A Study of the Biologic Behavior and Therapy of Extremity Sarcoma
 - A. Hemipelvectomy: Its Role in the Treatment of Extremity Sarcoma
 - B. The Use of Coumadin Anticoagulation as an Adjunct to the Surgical Treatment of Sarcomas
- Part XI. An Improved Technique for Radical Groin Dissection
- Part XII. The Role of Bacterial Infection in Cancer Surgery

Project Description:

- Part I. Study of the Biologic Behavior and Results of Surgical Therapy of Cancer of the Uterine Cervix
 - A. Surgical Treatment of Carcinoma of the Cervix

Objectives:

To evaluate extended surgical procedures for treatment of patients with recurrent cervical cancer and for selected patients with primary carcinoma of the uterine cervix.

Methods Employed:

Approximately 2100 patients with cancer of the uterine cervix have been referred to the Surgery Branch for evaluation. From this large group

550 were admitted for inpatient evaluation, 406 of whom underwent abdominal exploration with plans for definitive surgical therapy.

Of the patients explored, 163 were not candidates for exenterative surgery because of the presence of extrapelvic lymph node metastasis, intraperitoneal spread, or disease fixed to the bony pelvis. Eighty-two patients had relatively early cancers and could be effectively managed with a radical hysterectomy and pelvic lymphadenectomy. Thirteen of these patients had small radiorecurrent lesions, the remainder had primary cervical cancer.

One hundred sixty-one patients required either partial or total pelvic exenteration for control of their disease.

Major Findings

The major points of interest are summarized in the accompanying tables. The 5-year actuarial survival of those treated with radical hysterectomy and pelvic lymphadenectomy was 83.5%. Those patients with radiorecurrent or persistent disease survived at a rate similar to those with primary carcinoma. The presence of metastatic carcinoma in the pelvic lymph nodes adversely affected prognosis. The 5-year actuarial survival for those treated with exenteration of the pelvic viscera was 38%.

Significance to Biomedical Research and Program of the Institute:

The results obtained with extended surgical procedures are highly favorable and surpass those reported from other institutions (Annual Report on the Results of Treatment of Carcinoma of the Uterus and Vagina, 14th Volume). Extended surgical procedures are demonstrated to be effective in controlling advanced cervical cancer and are the only effective treatment modalities for patients with radiorecurrent cervical cancer. Selected individuals are offered vaginal reconstruction in an attempt to contribute to complete rehabilitation. The important ancillary studies generated by this program are detailed elsewhere in this report and include physiologic alterations following massive major surgery, dynamics of various forms of urinary diversion, the diagnostic advantages of "fine needle" cytology, and the efficacy of exfoliative cytology from urinary conduits in the early diagnosis of recurrent cancer.

Proposed Course:

The limits and the values of extended surgical procedures have been defined. Current plans call for a refinement of the diagnostic procedures to determine more specific and reliable criteria for operability and long-term cure. Although the Surgery Branch is no longer actively recruiting patients, thereby phasing out this project, significant numbers of patients with radiorecurrent disease are still being referred. These patients are included in the ancillary studies described above.

TABLE I
CARCINOMA OF THE CERVIX: ACTUARIAL 5-YEAR SURVIVAL
FOLLOWING TREATMENT WITH EXTENDED SURGICAL PROCEDURES

<u>OPERATION PERFORMED</u>	<u>PRIMARY CARCINOMA PELVIC LYMPH NODES</u>			<u>RECURRENT CARCINOMA PELVIC LYMPH NODES</u>		
	<u>Positive</u>	<u>Negative</u>	<u>Total</u>	<u>Positive</u>	<u>Negative</u>	<u>Total</u>
Radical Hysterectomy and Pelvic Lymphadenectomy	60.0%	91.3%	82.8% (69) *	85.7%	83.3%	84.6% (13)
Pelvic Exenterations (Partial and Total)	54.2%	52.2%	52.6% (66)	18.4%	35.6%	31.8% (95)
TOTAL (All extended surgical procedures without resection of bony pelvis)	56.4%	74.4%	67.6% (135)	30.5%	39.9%	39.1% (109)

*Numbers in parentheses represent the number of patients studied.

TABLE II
CORRELATION OF THE MEIGS' SURGICAL PATHOLOGIC STAGING WITH SURVIVAL
OF 162 PATIENTS WITH CARCINOMA OF THE CERVIX WHO UNDERWENT PELVIC EXENTERATION

PATHOLOGIC STAGING	5-YEAR CUMULATIVE PERCENT SURVIVAL		
	PRIMARY CASES	RECURRENT CASES	ALL CASES
A + A ₀	75% (5) *	43% (7)	60% (16)
B	56% (10)	0% (14)	33% (24)
C	31% (11)	49% (25)	44% (36)
D	60% (10)	17% (6)	44% (16)
E	43% (32)	18% (38)	30% (70)

*Numbers in parentheses represent total cases in each group.

TABLE III

PRIMARY CARCINOMA OF THE CERVIX: ACTUARIAL SURVIVAL (BY STAGE)
FOLLOWING EXTENDED SURGICAL PROCEDURES

PELVIC LYMPH NODES			
	<u>Positive</u>	<u>Negative</u>	<u>Total</u>
Stage I	75%	87%	84.3% (37) *
Stage II	60%	86%	80.0% (42)
Stage III	50%	75%	60.0% (10)
Stage IV	50%	32%	39.5% (37)

*Numbers in parentheses represent the number of patients studied.

Project Description:

Part I. Study of the Biologic Behavior and Results of Surgical Therapy of Cancer of the Uterine Cervix

B. Scalene Nodes as Indicators of Disseminated Cancer

Objectives:

To determine the instance of tumor dissemination, from primary pelvic cancer, to the scalene node bearing areas.

Methods Employed:

All patients with biopsy proven cancer of the structures of the female pelvis who are candidates for definitive curative surgery will have left scalene node biopsy. The procedure is carried out only in the absence of distal tumor spread. A generous pad of lymph node bearing tissue from the left sub-clavicular fossa is obtained from microscopic analysis. Using local anesthesia, this minor operative procedure can be carried out without complications.

Major Findings:

Sixty-nine patients with carcinoma of the cervix whose preoperative evaluation revealed no evidence of cancer spread beyond the confines of the pelvis and who were candidates for surgery had scalene lymph pad tissue removal. Eight of these patients were found to have unsuspected metastases to one of the lymph nodes in the subclavicular fossa. None of these patients had any evidence of pulmonary tumor or other metastases. They were, therefore, deemed incurable by surgery and a radical procedure avoided which would only have been palliative.

Significance to Biomedical Research and the Program of the Institute:

A significant number of patients developed metastatic disease at such a time interval following primary tumor treatment that there is the suggestion that the metastases were present preoperatively. No matter how complete and extensive the pretreatment evaluation, the surgeon must face this distressing situation only too often. The identification of metastatic disease from pelvic carcinoma to unsuspected anatomical areas adds another preoperative modality of determining what patient may be free of cancer metastases at the time of definitive surgery.

Proposed Course:

Continue study of all patients admitted for treatment of primary pelvic disease to rule out previously unrecognized metastases.

Project Description:Part II. The Surgical Treatment of MelanomaObjectives:

To determine the role of an aggressive standardized surgical approach to the therapy of malignant melanoma.

Methods Employed:

All cutaneous lesions or recent operative scars are excised with a minimum margin of 4 cm. of grossly normal skin. This necessitates skin grafting for closure in 90% of these wide local excisions. En bloc primary resection encompasses deep subcutaneous tissue and excision of fascia. Amputative surgery is usually indicated only for digital lesions. Resection of regional draining lymph node areas is carried out in continuity with the primary site excision when feasible. However, melanomas of the distal extremity are not resected in continuity with groin or axillary dissections. Bilateral lymphatic drainage areas are dissected when midline lesions are encountered. Radical deep groin dissections are carried out when the superficial groin nodes are found to contain tumor.

Major Findings:

In this long-term program, local control of a very distressing type of malignancy has been achieved in 93% of patients. However, failure to control distant metastases is the primary cause of a 60% overall mortality. Morbidity associated with wide local excision has been minimal. A method for axillary node dissection, with preservation of the pectoralis major muscle, has been devised to successfully reduce morbidity and disfigurement in these patients. In lower extremity melanomas, if superficial groin nodes are involved with tumor, deep groin node dissection is now carried out via a separate transabdominal approach. This has markedly decreased postoperative morbidity associated with poor healing of wound flaps and the distress of leg lymphedema.

Significance To Biomedical Research and the Program in the Institute:

The bizarre behavior of melanoma makes this one of the more poorly understood, but extremely interesting, cancers. Melanoma tissue harvested from patients treated in this study will be utilized in several experimental protocols involving in vitro cell culture of melanoma cells for use in in vitro cytotoxicity studies.

Proposed Course:

This continuing long-term program involves the cooperative efforts of several groups of laboratory investigators. Patients developing recurrent or metastatic disease will be candidates for an immunotherapy protocol which has been developed in conjunction with the Immunology Branch. Recent

emphasis on the histologic classification of primary cutaneous melanoma into nodular, superficial spreading, and melanoma developing in Hutchinson's freckle groups, has led to a review of the charts of 240 melanoma patients so that their primary lesions may be subclassified and their subsequent course correlated with the histology of their primary lesion. This continuing chart review is also considering the following questions:

1. What influence does the anatomic site of malignant melanoma have on subsequent survival following therapy?
2. What influence does sex have on survival following therapy?
3. Has prophylactic regional node dissection proved beneficial in the treatment of malignant melanoma?
4. Has therapeutic node dissection proved to be beneficial?
5. What factors relating to the primary lesion are important determinants of survival (histology, depth of invasion, size of lesion, etc.)?
6. To what extent has regional node involvement with melanoma influenced survival?
7. Have other forms of therapy utilized in the Surgery Branch provided significant benefit to patients?
8. What complications have followed surgical procedures? To what extent are they seen?
9. Are particular groups of patients worthy of special scrutiny because of unusual circumstances relating to the course of the disease (e.g., familial melanoma, disease associated with melanoma, prepubertal melanoma, multiple primary melanomas, etc.)?

Project Description:

Part II.

The Surgical Treatment of Melanoma

A. Surgical Staging of Melanoma Patients

Objectives:

To evaluate the extent of occult disease in pathologic Stage II melanoma patients with disease presumably localized to regional nodes.

Methods Employed:

Patients with Stage II melanoma which is malignant, as determined by histologic examination of excised tissue or aspiration cytology, undergo

comprehensive Surgery Branch diagnostic work-up. Surgical procedures, including fine needle aspiration, are used to evaluate accessible masses with emphasis on regional lymph nodes. Preoperative scalene node biopsy is carried out, as well as bone marrow biopsy. In patients whose primary lesion is so situated that therapeutic laparotomy will be required as part of the Surgery Branch treatment program for melanoma, intraoperative biopsy of the right lobe of the liver is carried out and lymph nodes excised from the lumbar peri-aortic and mesenteric areas. Stage II patients whose primary lesion is anatomically situated such that deep groin dissection would not be appropriate are managed as follows during the preoperative period: Scalene node biopsy, bone marrow aspiration and peritoneoscopy with needle biopsy of the liver. Diagnostic study of Stage II patients with an unknown chest lesion progress to thoracotomy, when necessary, to evaluate possible chest metastases. In clinical Stage I patients found to have positive regional nodes on frozen section during the course of a regional node dissection, laparotomy, if necessary for therapy, is carried out under the same anesthetic using the same staging procedure as described above. If superficial inguinal node involvement is first detected postoperatively, only after study of permanent sections of tissue obtained at superficial groin dissection, staging laparotomy and deep groin dissection is scheduled when the patient's clinical condition warrants. During this interval, bone marrow study and scalene node biopsy is carried out as indicated previously.

Major Findings:

Major findings cannot be predicted at this time. Aggressive surgical staging may reveal that significant number of patients thought to be pathologic Stage II will be found to have disseminated disease. This has been an experience in the staging of Hodgkin's disease, for example, where surgical procedures have led to a more accurate assessment of the anatomical extent of the disease and have directed application of therapeutic measures.

Significance to Biomedical Research and the Program of the Institute:

It is possible that surgical staging will show that more and more patients have less and less localized disease. Such findings would have implications regarding the surgical management of melanoma patients studied at the Surgery Branch of the NCI. In most patients who are found to have previously unsuspected disseminated disease on the basis of staging laparotomy or other diagnostic procedures, extensive surgery for control of primary lesions, and excision of regional nodes will not be indicated. Such patients will be designated Stage III-S (surgically staged) and will be considered prime candidates for chemotherapy or immunotherapy since they will carry a small tumor burden. Accurate staging and removal from the Stage II classification of patients found to be Stage III-S will eliminate those patients from the Stage II statistical categories and thus allow the real effectiveness of surgical procedures, such as wide excision of primary sites, therapeutic node dissections and prophylactic node dissections, to be evaluated in a group of patients who have truly localized disease. The immediate effect of this should be to show increased effectiveness of the surgical procedures currently used for Stage II disease.

Proposed Course:

Aggressive surgical staging of melanoma patients will be pursued on all appropriate patients. After 20 patients are studied as outlined above, the data will be carefully analyzed. Depending on the findings at that time, appropriate alterations in the study may be considered, e.g.:

1. Peritoneoscopy might be substituted for exploratory laparotomy in all patients.
2. Mediastinoscopy might be substituted for or used to supplement scalene node biopsy.

Project Description:

Part III. Malignant Melanoma: Screening of Potential Surgical Candidates with ⁶⁷Gallium Scintigraphy

Objectives:

To determine the accuracy and usefulness of the ⁶⁷Gallium scintigram in the detection of known and unknown metastatic disease in potential candidates for curative surgical treatment of biopsy proven melanoma.

Methods Employed:

Thus far, 24 sites in each of 48 Gallium scans on 44 consecutive patients with malignant melanoma (a total of 1152 sites) have been evaluated independently by two separate investigators without prior clinical information about the patient. Findings were compared with the results of extensive clinical diagnostic studies performed uniformly on all patients and wherever possible correlated with definitive histologic data. In several patients Gallium activity was assayed directly in selected samples of tumor and adjacent normal tissue obtained at surgery. Autoradiography was performed on these specimens as well.

Major Findings:

Of those sites for which histologic confirmation was available, almost all of the areas positive on Gallium scintigram were subsequently shown to contain malignant melanoma. The overall false positive rate was less than 5%. Those rare instances of false positive Gallium activity were associated with active inflammation or other local abnormality at the site. On the other hand, the overall false negative rate was 19%. This group of falsely negative scintigram sites accounted for the non-detection of 46% of the total number of melanoma containing sites (only 25% of those lesions greater than 2 cm. in size, 73% of those lesions equal to or less than 2 cm.). Autoradiography and direct tissue sampling studies confirmed the high affinity of melanoma tissue to ⁶⁷Gallium with tumor: background tissue ratios varying from 3:1 to 51:1.

Significance to Biomedical Research and the Program of the Institute:

The major factor in the relatively low cure rates for surgical treatment of malignant melanoma can be attributed to our inability to recognize clinically silent distant metastases. In this study $^{67}\text{Gallium}$ scintigraphy was shown to be a useful tool to detect those patients with clinically silent metastatic disease prior to surgery. Although some lesions were missed by this technique, the presence of an area of gallium uptake on the scintogram in an otherwise normal site was shown to be almost diagnostic of malignant melanoma at that site. The ability to detect such metastatic disease will not only provide the basis for avoiding unnecessary radical surgery in incurable patients, but also aid in accurate clinical staging to be used as a guide to the course of palliative immuno-, chemo-, and radiotherapy.

Proposed Course:

The $^{67}\text{Gallium}$ scintigram is now being used routinely to evaluate all surgical candidates for malignant melanoma. Long-term followup studies are presently underway on the group of patients studied so far in order to determine whether long-term followup will affect the above statistics. The use of the $^{67}\text{Gallium}$ scintigram to follow postoperative patients for the appearance of metastatic disease, and to follow Stage III patients in order to assay the progress of immuno-, chemo-, or radiotherapy is currently being evaluated.

Project Description:

Part IV. Fine Needle Aspiration Biopsy in the Diagnosis of Solid Tumors

Objectives:

To evaluate the accuracy of results obtained by fine needle aspiration of solid tumors in the diagnosis of malignancy; to evaluate the safety of fine needle aspiration. For over 15 years, Swedish investigators have used fine needle aspiration biopsy techniques to successfully diagnose tumors in breast, salivary glands, thyroid, lymph nodes, prostate, pelvis, and lung. Wide-spread acceptance of this technique has never been obtained in the United States. Studies carried out to date at NIH have indicated that the procedure is of definite value and can avoid open surgical incisional biopsy in many patients with solid tumors. If it can be demonstrated that the method is highly accurate and that interpretation is satisfactory using Papanicolaou's stain technique, it is possible that the procedure may gain favor in this country and lead to earlier diagnosis of solid tumors as well as assist in the management of metastatic disease.

Methods Employed:

A 22 gauge needle will be used to aspirate undiagnosed solid tumors. A special apparatus developed at the Surgery Branch will permit use of

disposable syringes to facilitate use of the technique. Material obtained by aspiration will be smeared on microscopic slides and stained by the Papanicolaou technique. The syringe and needle is then rinsed with saline and this material put through a millipore filter and stained with the Papanicolaou stain. The slides are read by cytologists at NIH and are available for training of cytologists at NIH and other institutions in the United States, in the interpretation of aspiration biopsy smears.

Major Findings:

Aspiration biopsy has been carried out on over 250 solid tumors. Diagnoses established by cytological examination have correlated well with diagnoses made upon histologic examination of excised tissue. Accuracy has been 95% in diagnosing tumor in lymph nodes and 93% in diagnosing tumor in subcutaneous nodules. False positive cytologic reports have not occurred. False negative reports occur in about 10% of aspirations. It is anticipated that this will be reduced as familiarity with aspirating technique is gained. In addition, establishment of an aspiration clinic is planned so that prompt examination of the aspirate can be carried out with re-aspiration if necessary. Cells aspirated from tumors will be placed in tissue culture for possible use in future immunotherapy programs and for use in cytological grading of tumors. Fine aspiration technique will be used as part of the newly established breast cancer control program at NIH. Aspiration of breast masses will help identify those patients, approximately 90%, whose breast lumps are benign and in whom outpatient excision under local anesthesia can be carried out. Thus unnecessary trauma and expense to the patient can be avoided. As the cytologists have been presented with increasing amounts of material, interpretation of the Papanicolaou smears have become increasingly accurate. Intra-operative aspiration of nodules encountered during surgical dissection in two patients has enabled more accurate planning of the extent of the operative procedure by the surgeon.

Significance to Biomedical Research and the Program of the Institute:

Earlier diagnosis of tumors is felt essential for application of therapeutic measures and improved therapeutic results. It is anticipated that fine needle aspiration may provide a fast, accurate method of cancer diagnosis in over 90% of tumor masses aspirated. The avoidance of surgical incisional or excisional biopsy should accelerate establishment of diagnosis and treatment of the patient. Cost should also be significantly reduced. The technique may be applicable to screening clinics and may represent a significant advance in the early diagnosis of cancer.

Proposed Course:

Aspiration with large numbers of solid tumors in various anatomical locations is being carried out. Efforts will be made to expand the use of the technique to physicians throughout the National Cancer Institute. In conjunction with the Cytopathology Department, plans are underway to give instructional courses to cytologists, outside the NIH, and interpretation of the smears.

Project Description:Part V. Medullary Extension of OsteosarcomaObjectives:

To determine the extent of clinically unappreciated intramedullary extension of osteosarcoma in clinical cases.

Methods Employed:

Amputation specimens from the clinical population of the Surgery Branch, NCI, are examined in cooperation with the Laboratory of Pathology. Radiographic (preoperative) and gross pathologic characteristics were correlated. Microscopic examination of specimens is performed.

Major Findings:

Radiologic and gross pathologic extent of tumor have been found to be similar in this series. Unappreciated medullary extension of osteosarcoma was absent. Dissemination of foci of osteosarcoma within the medullary cavity, remote from the main tumor mass, separated by so-called "skip areas", have not been found.

Significance to Biomedical Research and the Program of the Institute:

Varying investigators have reported conflicting results as to survival when osteosarcoma is treated by amputation. Many centers report equal survival rates with transmedullary (conservative) amputation to those obtained by radical amputation. The advocates of radical amputation maintain that isolated foci of tumor, beyond uninvolved "skip areas" in the medullary canal, are often present and are left following conservative amputation. A systematic pathologic study of clinical specimens could help to elucidate whether the radical approach, with its increased morbidity, has a scientific basis.

The results of this study, and one other study in the literature, suggests that there is no pathologic basis for advocating radical amputation in osteosarcoma. Coupled with similar survival rates, these findings support the view that transmedullary amputation, with its vastly lessened morbidity, may be the procedure of choice for selected osteosarcomas.

Proposed Course:

Continued comparison of the microscopic extent of tumor found in excised bones and comparison with the extent determined radiographically will be pursued for determination of the feasibility of a study of the role of transmedullary amputation in patients with osteosarcoma in the distal portion of long bones.

Project Description:Part VI. Evaluation of Uretero-ileostomy Following Urinary DiversionObjectives:

Despite the wide acceptance of the ileal conduit, it is recognized that patients following this procedure have a significant incidence of complications related to the upper urinary tract. For this reason, such high risk patients have required constant surveillance of the urinary transport system. This is usually monitored by the frequent performance of excretory urography. Repeated use of such radiographic procedure carries with it the inherent risks associated with cumulative irradiation as well as idiosyncratic or allergic reactions. Since patients undergoing conduit diversions for benign and malignant disease can now expect a relatively long period of survival, a safe, accurate procedure to regularly evaluate the status of urinary transport system would be useful. The radioisotope renogram appears to satisfy these criteria, although its accuracy has not been formally tested in patients with ileal conduit. The present study was designed to evaluate if the radioisotope renogram was as reliable as the excretory urogram in detecting urinary tract obstruction in patients with ileal conduit diversion.

Methods Employed:

Patients who have undergone total pelvic exenteration and ileal conduit urinary diversion for cancer of the uterine cervix at the Surgery Branch of the National Cancer Institute will be studied. Renograms and scintiphotos will be obtained using an Anger scintillation camera. Prior to the procedure each patient will receive 10 cc/kg of water by mouth which should assure a urine flow of 1.5 to 2 cc/min. All renograms and scintiphotos will then be correlated with the patient's clinical state and her excretory urogram.

Major Findings:

The first important findings concerned the proper position to perform renography in patients following urinary diversion. It was discovered that if the customary supine position was utilized for renography, a significant number of patients had an obstructive renogram pattern. If, however, the renogram was repeated in the sitting position, the pattern obtained was either normal or corresponded to the patient's excretory urogram. After the initial 15 patients had been studied in both the supine and the sitting position, all other renograms were obtained in the sitting position. An excellent correlation existed between the degree of pyelectasis and/or caliectasis and the renogram as measured by the time from injection of the isotope to the point of maximum count rate over the kidney (T-max). Although a significant difference existed between T-max values of normal appearing kidneys and those with minimal dilatation, there was some overlap between

these groups. Since ureterectasis alone, and some renal units with minimal hydronephrosis, were not associated with an abnormal T-max, the anatomic abnormalities seen by urography may not be indicative of a functional obstruction. All patients with moderate or marked hydronephrosis had abnormal renograms.

Significance to Biomedical Research and the Program of the Institute:

The accuracy, safety, and convenience of renography make it ideally suited for the long-term monitoring of patients with ileal conduit urinary diversion. We feel that patients with ileal conduit urinary diversion can be safely followed for long periods of time with repeated renograms rather than every 6 months to 1 year excretory urograms. In addition to the renogram the patient should have the customary studies for urinary function, urine culture, as well as a KUB x-ray to rule out calculus formation.

Proposed Course:

Patients following ileal conduit urinary diversion will continue to have renography performed and additional patients added to the study to determine the accuracy of this procedure.

Project Description:

Part VII. Axillary Dissection with Preservation of the Pectoralis Major Muscle

Objectives:

Because of the severe disability resulting from the currently used techniques of axillary dissection, a re-assessment of the operation was undertaken. Heretofore, two techniques of gaining access to the axilla were used, either excision of the muscle, which greatly weakens the upper extremity, or transection and resuture of the muscle, which partially devascularizes and denervates the muscle; it also requires prolonged immobilization after the operation and often results in residual limitation of motion and weakness of the extremity.

Methods Employed:

To devise a technique of access to the axilla without transection of the pectoralis major muscle, experiments were conducted using normal volunteers, cadavers, and finally in patients having axillary dissections. It was found that a semilateral position of the patient with the arm adducted across the chest proved to give adequate exposure of the axilla contents without section of the muscle and without compromise of the goals of the operation. This unique position of the patient in turn required the development of a new technique of executing the operation.

Major Findings:

Thus far, axillary dissection utilizing this position and technique has been completed in 52 patients. With the exception of 2 in whom partial resection of the innervation of the muscle was necessary because of bulky metastatic tumor deposits, the neurovascular bundle to the pectoralis major was preserved. The average postoperative follow-up of the patients is 36 months at present. In spite of the fact that 24 patients had axillary lymph nodes involved with metastatic tumor and in 13 of these the tumor had extended through the node capsule into the axillary areolar tissue, no recurrences have developed. All patients have had per primum healing of their wounds and have regained full motion of the extremity. In addition, edema of the upper extremity commonly seen after the conventional technique of axillary dissection utilizing excision of the pectoralis major muscle has not occurred in these patients.

Significance to Biomedical Research and the Program of the Institute:

Resection of the axillary lymph nodes is an established aspect of the treatment of tumors of the extremity and adjacent chest. The technique of the operation described has proved to give a superior cosmetic and functional result in our patients and has been of particular benefit in those patients who have required bilateral axillary dissections, most of whom were male laborers.

Proposed Course:

A detailed illustrated description of the operation has been published. The major cosmetic and functional advantages offered by this approach to axillary dissection suggest that it will be adopted as a standard technique.

Project Description:Part VIII. Extended Deltopectoral Flap Reconstruction of Anterior Oral DefectsObjectives:

To develop a direct, easily accomplished, and reliable means of reconstruction of defects of the chin, anterior floor of mouth, and tongue that offers a superior cosmetic and functional result to that achieved with conventional techniques of reconstruction.

Methods Employed:

To date, no satisfactory means of reconstructing defects in the chin, anterior floor of mouth, and tongue has been devised. The cosmetic deformity resulting from resection of massive defects in this region and the functional disability e.g., continuous drool, impairment of speech, prevention of mastication, and

pulmonary aspiration of saliva led to an assessment of this heretofore unsolved problem. A flap having its base in the sternal region and extending to a tip in the posterior axillary fold was developed for use in the reconstruction of these defects. The flap has been perfected so it can be raised and rotated without loss of viability of the tip and with minimum inconvenience to the patient.

Major Findings:

The extended deltopectoral flap has been utilized in 13 patients thus far, including 5 who had virtually complete loss of the free tongue. It has allowed complete restoration of speech and swallowing in each case. The technique represents the first cosmetically and functionally satisfactory means of restoration of tissue in such defects.

Significance to Biomedical Research and the Program of the Institute:

Impairment of function and the distortion of the appearance of patients having massive resections of tumors involving the chin and anterior tongue region has constantly presented a challenge to the members of the Surgery Branch as well as head and neck surgeons elsewhere. The use of this technique of reconstruction will markedly shorten the hospitalization time of these patients following removal of such malignancies, and allow a rapid and more complete return to their community with elimination of the nursing care required heretofore after such operations.

Proposed Course:

Improvements in the technique of reconstruction utilizing this flap will be made so as to allow its use both at the time of resection of the primary tumor and in those patients who had resection of the tumor as sometime in the past and have residual, functional and cosmetic deficits. In addition, this flap has been used in combination with the extended shoulder flap in several patients who had full thickness losses of the face that would have been virtually impossible to construct with currently available techniques. Further improvements in the technique of reconstruction with such flaps should relieve the reticence of the surgeon to resect massive malignancies of the head and neck region which, though technically feasible, would create distortions of appearance and function that would deter the patient from accepting the operation.

Project Description:

Part IX. A Study of the Results of Surgical Treatment and Effects of Adjunctive Therapy in Cancer of the Head and Neck

Objectives:

1. To determine the feasibility of an aggressive surgical approach to irradiation or surgical recurrent malignancies of the head and neck areas.

2. An evaluation of adjunctive chemotherapy used before surgery and its long-term intermittent use after surgery.
3. To determine the effectiveness of a combined intracranial facial approach to cancer of the paranasal sinuses.
4. To determine the prevalence, site of origin, and rationale of treatment of unknown primary disease metastatic to head and neck areas.
5. Evaluation of new methods of surgical approach and reconstruction in surgical treatment of head and neck cancer.
6. The clinicopathological correlation of epidermoid carcinoma of the head and neck area.

Methods Employed:

Following extensive preoperative clinical, pathological, and roentgenological studies, standard and new radical surgical procedures are carried out.

(a) In patients with Stage II, III, and IV epidermoid cancers of the oral cavity treatment with methotrexate is used for 2 to 3 weeks before surgery, and after surgery will be used on a long-term intermittent basis. Surgery will involve wide resection of the primary tumor with unilateral or bilateral neck dissection. An effort will be made to increase survival and decrease the incidence of local recurrence with this combined chemotherapy and surgery approach.

(b) In patients having paranasal sinus cancer, either primary or recurrent which suggests roentgenographically or clinically that the cribriform plate will be removed at the time of surgery, our approach will be from an intracranial and combined facial surgical incision which will permit en bloc removal of the tumor and at the same time afford protection of the surrounding vital structures.

(c) In patients requiring surgery to control recurrent disease following previous therapy, treatment will be combined pre- and postoperatively with a chemotherapy approach. During our continuing studies for inpatients with head and neck cancer some 70-80% of the patients screened for admission have been failures with previous therapy, usually irradiation. The actual incidence of patients admitted and treated who have been previous failures is 71%. By using chemotherapy before and after surgery it is anticipated that an improvement in survival results will be realized.

(d) In patients requiring large resection for treatment of their cancer immediate plastic reconstruction will be carried out using flaps of tissue from the pectoral or shoulder areas. These flaps will be used singly or in combination at the time of the resection.

Major Findings:

Over the past 2 years, 27 patients have been entered on a pilot combined methotrexate and surgery protocol. No complications have been encountered.

The majority of the patients have demonstrated a marked reduction in the size of their tumor and metastatic neck nodes during the 2 week pre-surgery treatment period. These encouraging results have led to the formulation of a prospective comparison of adjunctive methotrexate and radiotherapy in patients receiving surgical treatment of head and neck cancer.

The combined intracranial facial operation for cancer of the paranasal sinuses has been performed in 53 patients to date. A review of the results revealed that of the patients treated, 19 were dead and 24 were living for a time interval of 4 months to 11 years follow-up. The operation has been extended to include patients showing some involvement of the cerebral meninges. All of the patients who have thus far been approached in this manner have had extensive disease and have not been amenable to treatment in any other fashion. Any survival at all is indeed promising, but more patients and more time is necessary before the procedure can be recommended for routine use. The advantages of the combined procedure are that it involves (1) evaluation of intracranial disease, (2) protection of the brain, (3) avoidance of spinal fluid fistulas, (4) more adequate hemostasis, (5) facilitation of resection, and (6) en bloc resection.

Unknown primary lesions with neck metastases is not a frequent occurrence and accumulation of a series of significant numbers is difficult. The eventual discovery of the primary lesion has usually been in the nasopharyngeal sinus area, but 3 cases have been from the thyroid and 1 from the kidney. In the past year, 6 new cases have been admitted to this study.

Significance to Biomedical Research and the Program of the Institute:

These locally resistant, usually recurrent head and neck lesions have long been a problem to oncologists. We are attempting to correlate aggressive surgical exploration of these lesions with survival and the mode of tumor dissemination. Careful environmental background reviews reflect the close relationship between the Surgery Branch and the Dental Prosthetic Unit allowing a comprehensive evaluation of the indications for plastic reconstruction of facial deformities and evaluation of newer prosthetic materials.

Proposed Course:

The extent of surgical excision cannot be much increased in its scope beyond that which we are now able to perform successfully. More patients are necessary before the combined cranial-facial approach to paranasal sinus cancer can be recommended for routine use. Both procedures are based on sound experimental and clinical observations and final evaluation should prove rewarding. Pilot studies are underway to determine the feasibility of intensive preoperative medication in patients with disease which has locally extended beyond that which can be cured by conventional means.

Project Description:Part IX.A Study of the Results of Surgical Treatment and Effects of Adjunctive Therapy in Cancer of the Head and NeckA. A Retrospective Computer Analysis of Patients with Head and Neck Tumors Treated Surgically at the NIHObjectives:

One of the least refined areas of cancer biology concerns the ability of the physician to assign prognostic significance to laboratory and clinical data. Presently clinical and histologic staging are the only reliable criteria now utilized to predict patient survival and even with these criteria there is considerable variability. With the multiple diagnostic and evaluative tests now available, an effort is being made in this study to determine other predictive factors relative to survival in the head and neck cancer patients. Available evidence indicates the defense mechanism which might be immunologically mediated in the host might be of prime importance in achieving long-term survival from many different malignancies. There has been considerable recent interest in the prognostic significance of lymph node reactivity of patients with carcinoma of the breast. Correlative value has been ascribed to bilaterally palpable axillary nodes and the cellular structures of nodes examined. A similar study is underway with patients treated surgically for cancer of the larynx.

Since cancer of the head and neck represents a variety of disease, the patients have been assigned to traditional categories based on location of the primary tumor for the purpose of comparing our survival data with other series. The first group to be studied were those patients with cancer of the larynx and hypopharynx. Patients with tumors of the oral cavity, nasal cavity, paranasal sinuses, salivary glands, skin, and unknown primary sites will be studied subsequently.

Methods Employed:

A detailed retrospective study of all head and neck cancer patients treated surgically at the NIH will be undertaken. To date 84 patients with cancer of the larynx have been studied. Approximately 200 clinical and histological variables extracted from the records of each patient were submitted for computer analysis using the data processing program of the cancer end results section.

Major Findings:

The majority of patients studied had either very extensive primary disease or radiation recurrent cancer. Of the 84 patients, 44 had intrinsic primary laryngeal tumors, 20 had extrinsic primary disease, and 20 patients had radiation recurrent or persistent tumors unresponsive to therapy. The involvement of cervical nodes was taken as the prognostic standard against

which all other variables were compared. Intrinsic lesions with negative nodes had a 71% 5-year survival while positive nodes indicated a cumulative 5-year survival of 33%. As expected, positive nodes were more likely to occur with extrinsic lesions (63% vs. 24% for intrinsic lesions) and resulted in a drop in 5-year survival from 49% for patients with negative nodes to 30% with positive nodes. Other less recognized criteria were equally significant. Of the 13 patients with cytologically positive intra-operative wound washings only 2 were alive at 5 years. The likelihood of cure was considerably greater in those patients who had either a unilateral or bilateral elective neck dissection (19 patients, 78% survival) than those patients who had a neck dissection for clinically positive nodes (30%) or in those who had clinically negative nodes and no primary neck dissection (46%). If there was a positive intra-operative frozen section margin for which additional tissue had to be taken to assure a clear margin, the total cumulative 5-year survival dropped from 54% for those with initially clear margins to 13% for those with at least one positive frozen section margin. Of the 11 patients with microscopically positive margins on permanent section the 5-year survival was 28%. Multiple preoperative variables were likewise statistically evaluated and found to be significant in predicting ultimate survival. For example, in patients with preoperative serum albumin levels of less than 3 mgm%, the cumulative 5-year survival was 29% while those with albumin levels of 3.9 mgm% or greater had a 75% likelihood of living 5 years.

Significance to Biomedical Research and the Program of the Institute:

It is apparent that there are numerous clinical tests and other less utilized variables which have prognostic significance in predicting long-term survival in patients treated surgically for cancer of the larynx. Hopefully, similar variables will be identified in other head and neck cancers as this project proceeds.

Proposed Course:

The remainder of the patients with head and neck cancer treated at the NIH will be studied retrospectively using the same computer program for data processing.

Project Description:

Part X. A Study of the Biologic Behavior and Therapy of
Extremity Sarcoma

Objectives:

A review of patients operated on by the Surgery Branch of the National Cancer Institute for sarcomas of bone as well as soft tissue sarcomas primarily involving the trunk and extremities was undertaken. The questions of survival statistics, incidence of regional node metastases, and the incidence of

local recurrence following surgery were of particular interest. Also of interest were the results of therapy at the National Cancer Institute as compared to the published results country-wide.

Methods Employed:

Case records of patients operated on by the Surgery Branch from 1953 until December 31, 1968, were reviewed. Patients studied were those who underwent definitive surgery at this institution.

Major Findings:

Sixty-two patient records were reviewed in detail. The survival statistics for 2 years and greater demonstrated the following: rhabdomyosarcomas, 3 of 8; liposarcoma, 2 of 4; undifferentiated sarcomas, 1 of 4; fibrosarcomas, 7 of 14; chondrosarcomas, 8 of 11; synovial sarcomas, 1 of 1; mesenchymal sarcomas, 1 of 2; osteogenic sarcomas, 2 of 16 (1 of these 2 was a parosteal sarcoma); leiomyosarcoma, 1 of 1.

This gave an overall survival rate of 26 out of 62 patients.

In this 15 year period there were 21 hemipelvectomies performed and 9 interscapular thoracic amputations performed. The mortality from this extensive surgery was minimal, only 1 patient of these 62 died in the post-operative period and that was a patient who died of renal failure after a combined total pelvic exenteration-hemipelvectomy.

There were 4 local recurrences, 1 following radical local excision, 2 following interscapulothoracic amputation, and 1 following hemipelvectomy. These local recurrences were all in the patients who had massive local lesions.

Significance to Biomedical Research and the Program of the Institute:

Survival statistics and local recurrence rates were comparable to published reports. Of particular importance was documentation of the extremely poor outlook for the patient with an osteogenic sarcoma, with the survival being 10% or less at this institution. The other common lesion treated here with a poor prognosis was fibrosarcoma, with a 50% survival rate greater than 2 years. These data have suggested that we can select patients on the basis of their histological diagnosis and the size of the lesion to represent very poor risk patients and include them in some type of an adjuvant protocol. At present only the patients with metastatic cancer have been treated by immunotherapy, but certainly patients with an expected survival of only 10% would be an excellent group to attack aggressively with immunotherapy. Since osteogenic sarcomas presumably do contain a common tumor specific transplantation antigen aggressive immunotherapy seems indicated. In addition, patients with osteogenic sarcomas or large soft tissue sarcomas would be acceptable for aggressive adjuvant chemotherapeutic protocol.

Proposed Course:

The patients who have been operated on since January 1, 1969, will be reviewed to keep the study up to date. This data is being made available to the National Sarcoma Task Force for country-wide evaluation of patients with soft tissue sarcomas. Further evaluation of rehabilitation of patients who have been amputated is being undertaken to better evaluate patients preoperatively and to assist them in postoperative convalescence. The data is being looked at closely from the standpoint of seeing with which tumors we can avoid radical amputative surgery or particularly in patients whose prognosis is grim.

Project Description:Part X. A Study of the Biologic Behavior and Therapy of
Extremity SarcomaA. Hemipelvectomy: Its Role in the Treatment of
Extremity SarcomaObjectives:

To develop a technique of hemipelvectomy that minimize the skill required, the operative time, and blood loss entailed.

Methods Employed:

As a result of the reluctance of surgeons to perform hemipelvectomy when indicated, because of the technical skill required in the execution of the operation, lesser procedures which lead to a higher recurrence rate and mortality rate are being employed on a widespread basis. In an effort to improve the survival rate with such patients, we have recently completed a study of the operation in cadavers and living patients and devised a technique that is within the skills of the average general and orthopedic surgeon.

Major Findings:

The conclusion of an assessment of the problem was that the most difficult aspect of hemipelvectomy as conventionally performed was the deep pelvic dissection. We then found that the relatively limited exposure to this anatomically complex and vascular area afforded when the operation is performed with the patient in the supine position is obviated with the patient in the lateral decubitus position. The addition of the Trendelenburg position then afforded additional exposure because of the gravitational shift of the viscera into the upper abdomen. In addition, simultaneous access to the anterior and posterior aspects of the pelvis resulting from this patient position, as well as the perineum when the extremity was elevated, allowed the rapid execution of the operation, particularly in those instances in which uncontrollable bleeding was encountered during the deep pelvic dissection.

After studies in cadavers suggested that these concepts should be assessed in living patients, the operation was so utilized with such great success that it was adopted as the standard technique by the Surgery Branch.

Significance to Biomedical Research and the Program of the Institute:

The alarming number of lower extremity malignancies referred to this Institute after recurrence with inadequate operation because of the reluctance of the referring physician to perform hemipelvectomy indicates the need for an easily mastered technique for the operation. The technique developed should have a favorable effect on the surgeon's willingness to perform this operation as the initial procedure, thus giving the patient the maximum opportunity for cure. In addition, the utilization of this technique by the Surgery Branch has shortened the operative time and the blood loss entailed in the operation, and allowed the safer performance of these operations in relatively poor risk patients.

Proposed Course:

A detailed and illustrated disclosure of the technique for publication in a surgical periodical is in progress. In addition, a motion picture depicting the advantages of the operation as well as a detailed step by step conduct of the operation in a patient has been completed and submitted to the Film Library of the American College of Surgeons to make it available for distribution to general and orthopedic surgeons.

Project Description:

Part X. A Study of the Biologic Behavior and Therapy of Extremity Sarcoma

B. The Use of Coumadin Anticoagulation as an Adjunct to the Surgical Treatment of Sarcomas

Objectives:

Patients with extremity sarcomas thought to be curable by surgery have a poor survival, approximately 20 percent at five years. Perioperative anticoagulation in animal tumor systems has demonstrated a marked increase in survival or a decrease in incidence of pulmonary metastases. Warfarin anticoagulation is to be tested in the clinical setting to see if it will improve the survival of patients with extremity sarcomas amenable to surgical amputation.

Methods Employed:

Patients with histologically proven sarcoma thought to be curable by amputation will be admitted to the study. Evidence of metastatic disease or any contraindications to anticoagulation will exclude the patient from the study.

The patients are begun on oral warfarin one week prior to surgery. Anti-coagulation is to be monitored by the standard prothrombin time, prothrombin-proconvertin levels, and specific assay of plasma factors VII-X, VIII, and IX. The prothrombin time is to be maintained 6-7 seconds above control (10.0 - 11.5 second range). The P&P test is to be maintained in a range of 12.5-25%. Drugs known to interfere with the metabolism or uptake of warfarin are to be avoided.

Major Findings:

Twenty patients have been included in the protocol to date. Twelve of twenty patients are alive and free of metastases at this time. There have been 14 osteogenic sarcomas operated on, seven of which are alive at periods of 3 mo., 8 mo., 11 mo., 15 mo., 2-1/2 years, and 4 years. Six other patients with various histologic types of sarcomas have been anti-coagulated, 5 of which are alive at periods of 5 months, 7 months, 23 months, 2-1/2 years, and 3 years. Six major wound problems have been attributed to anticoagulation. Bleeding complications were encountered in 8 patients necessitating stopping the Warfarin anticoagulation for at least a short period of time. All of these patients were over anti-coagulated according to our criteria.

Significance to Biomedical Research and the Program of the Institute:

A very high mortality rate continues to occur in patients with sarcoma, despite radical amputative surgery. For this reason, effective adjunctive therapy must be searched for in this disease. It is hoped that anti-coagulation will improve the results of surgical treatment for sarcoma patients. A greater understanding of the mechanism involved in tumor metastases is also sought.

Proposed Course:

Patients will be randomized to either control or Warfarin therapy instead of being compared with historical controls. The level of anticoagulation will be followed much more closely with the assistance of the Hematology Section of the National Cancer Institute. It is felt that closer monitoring can reduce the hemorrhagic complications that have been encountered.

Project Description:

Part XI. An Improved Technique for Radical Groin Dissection

Objectives:

Standard techniques of radical groin dissection entail significant immediate and long-term morbidity. The immediate morbidity is primarily due to necrosis of the skin flaps leading to infection of the operative wound, thrombosis of the femoral vessels, and thromboembolism. Prolonged hospital

stays occur in approximately 1/2 of patients having this operation. The long-term morbidity consists of persistent and incapacitating lower extremity edema with limitation of motion and skin ulceration. This study was undertaken to develop an improved technique of radical groin dissection which would obviate these morbidities.

Methods Employed:

Through a trial of all existing techniques and study of the anatomical factors involved in radical groin dissection, a technique was devised that utilizes two incisions. An oblique incision paralleling the inguinal crease is used to dissect the superficial inguinal lymph nodes. A second, midline incision is used to dissect the pelvic lymph nodes. In this technique, the circumflex iliac and inferior epigastric vessels are preserved.

Major Findings:

Using the technique described, thus far 35 patients have had radical groin dissections. Eighteen of these were unilateral and 17 were bilateral radical groin dissections. The immediate morbidity due to flap necrosis has been minimal, and in no case has delayed the ambulation of the patient. The long-term result has likewise been gratifying. None of these patients has developed extremity edema or morbid skin changes.

Significance to Biomedical Research and the Program of the Institute:

An important contribution to the treatment of cancer patients would be the reduction of the morbidity associated with radical groin dissection. This operative technique appears to have achieved this goal. This is reflected in decreased postoperative hospitalization time and concomitant or rapid return to preoperative functional capacity of the patients who had radical groin dissection by this operative approach.

Proposed Course:

Continued study of this technique is in progress with an attempt to further improve it and adopt it for all situations requiring radical groin dissection.

Project Description:

Part XII. The Role of Bacterial Infection in Cancer Surgery

Objectives:

To further substantiate findings in the previous years of the evaluation of the operative wound infection rate and its relation to the "prophylactic" use of antibiotics following extensive cancer surgery.

Methods Employed:

In the past year the immediate admission and repeated preoperative culturing of all patients' nose, skin, and tumor allowed patients to be classified into either carrier or noncarrier status of hemolytic staphylococcus aureus coagulase-positive organisms. By bacteriophage typing, through the cooperation of the Clinical Pathology Department, a correlation could be drawn between the patient's preoperatively carried organism and that carried postoperatively, either asymptotically or related to a postoperative infection. In previously reported studies patients undergoing radical cancer surgery had, on a double blind basis, either 10 days of chloromycetin postoperatively or a placebo. A subsequent study entailed the use of 7 days of chloromycetin postoperatively in all patients, while on a double blind basis the patients received preoperatively either 3 days of placebo or 3 days of antibiotic. The current protocol utilized intravenous antibiotics before, during, and 7 days following the operation. Following 2 separate studies, which have been reported, antibiotic therapy has been used prophylactically in a therapeutic manner on patients undergoing cancer therapy. These patients have been carefully observed for wound infection and any possible correlation to their "carrier status" of pathogenic organisms.

Major Findings:

The previous annual reports detail the specific results of both double blind antibiotic studies. In brief, the infectious complication rate following extensive cancer surgery was lowered from 54% to 14% by the use of pre- and postoperative antibiotics rather than placebo. An 8% infection rate occurred when the intraoperative use of antibiotics was employed in addition to pre- and postoperative coverage.

The overall results for the last 16 years can be seen in the accompanying table. The 1960 rate was elevated to 32% in that this was the year of the original study, whereas 1959 was lowered somewhat because of the discontinuance of the use of penicillin and streptomycin and the selection of antibiotics was left to the discretion of the surgeon.

17 YEAR SUMMARY OF INFECTION RATES AND
STAPHYLOCOCCAL INCIDENCE FOLLOWING EXTENSIVE CANCER SURGERY

Year	Extensive cancer operations	Infection rate per cent	Per cent of total infections that were staphylo- coccal
1956....	128	28	35
1957....	139	31	42
1958....	145	30	59
1959....	130	22	69
1960....	144	32	74
1961....	115	13	73
1962....	132	10	72
1963....	137	15	70
1964....	131	7	51
1965....	132	6	36
1966....	141	5	22
1967....	128	6	17
1968....	209	8	14
1969....	217	10	8
1970....	132	11	7
1971....	197	13	4
1972....	208	19	2

During the past 17 years, 1,813 extensive cancer operations have been performed by the Surgery Branch of the National Cancer Institute. During the early part of this study the infectious complications ranged from 22% to 31% during a time when penicillin and streptomycin were used somewhat routinely at the discretion of the surgeon. The results of a double blind antibiotic-placebo study revealed an infection rate of 54% when a placebo was used and 14% when routine 10 day postoperative antibiotic therapy was instituted. A subsequent double blind study showed that by giving drug for 3 days preoperatively and 7 days postoperatively antibiotic therapy resulted in a 17% rate of infectious complications. With continued use of antibiotic prophylaxis, while continuing the same type of extended operative procedures, the infection rate was 10% and 15%. Chloramphenicol, 2 gm. every 6 hours, was the antibiotic used and there were no significant complications referable to drug toxicity and only 5 severe infectious complications referable to loss of staphylococcal sensitivity to the drug.

Over the past years the percentage of total infections that were hemolytic staphylococcus aureus DNA-ase positive in origin progressed from 35% in 1956 to 70-75% in 1961-64, and dropped to 36% in 1965 and 14% in 1968 and was 2% in 1972. At the same time, the incidence of patients who were carriers of staphylococci on admission to the hospital was 22% and has remained at that approximate level. In the past, patients who carried

staphylococci into the operating room, usually asymptotically, ran at 38% increased rate of infection over noncarriers. Ninety-three percent of these patients who subsequently become infected as an operative complication will become infected with the same phage type organism that they carried into the operating room. This suggests that self-infection may play as important a role in infectious complications as does cross-contamination. Of greater importance at this time is the alarming increase in the complications associated with *Pseudomonas* and *Klebsiella* and *E. Coli* infections. So frequently death is the result of a septicemia from these organisms. They are much more of the problem today both in prevalence and in morbidity and mortality than the staphylococcus. The gradual increase in wound infections which we are again noting, from a low of 5% in 1966 and now 19% in 1972, is in part due to newly emergent organisms which have in the past been uncommon as a threat. However, the chief reason which has not yet been demonstrated by a controlled study is apparently related to the lack of uniformity in prophylaxis in the administration of prophylactic antibiotics for patients undergoing extensive cancer procedures. Justified trend in the conservative use of antibiotics in patients undergoing general surgical procedures is seemingly leading to increased morbidity in these cancer patients. In addition, the trend on the part of the resident staff, who are from general surgical residencies without significant cancer orientation, is to use antibiotics rather sparingly. This is a procedure well documented in general surgical cases in contrast to our experience citing the need for "prophylactic" antibiotics in cancer patients.

Significance to Biomedical Research and the Program of the Institute:

When postoperative morbidity and mortality are increased due to complications related not directly to the cancer but due to infection, the overall evaluation of surgery and its place in cancer therapy is markedly altered. Bringing an alarming infection rate under relative control has decreased morbidity and mortality as indicated in the previous paragraphs. In a hospital such as this where rather elaborate facilities are available for close patient surveillance, studies such as this can be done which can be of significant value to the practicing surgeon. The fact that the total infectious complication rate seems to be rising may be related to the reluctance on the part of the clinician to use Chloramphenicol which has been shown over the years to be the drug to which the hospital organisms are most susceptible. Because of the medicolegal aspects related to Chloramphenicol administration, the resident staff use this antibiotic only on direct insistence on the part of the senior staff. The continued decrease in the staphylococcal infection complication rate is consistent with findings in most hospitals throughout the country.

Proposed Course:

The indications for "prophylactic" antibiotic usage when carrying out the present clinical investigative studies within the Surgery Branch are unequivocal. Close surveillance of carrier patients and personal carriers within

the hospital is being continued. It is anticipated that continuation of the surveillance program will not only lessen the so-called epidemic infectious outbreaks occasionally seen on all surgical wards but will allow more rapid and adequate control when such does occur. It is proposed that the critical period of antibiotic coverage is at the time of the operative procedure itself and that constant infusion and drug level maintenance of antibiotics during this period is mandatory in cancer surgery.

Publications:

Ketcham, A.S.: A surgeon's approach to the patient with advanced cancer. In Schoenberg, B.S., Carr, A.C., Peretz, D., and Kutscher, A.H. (Eds.): Psychosocial Aspects of Terminal Care. Columbia University Press, 1972, pp. 88-97.

Soloway, M.S., Larsen, S.M., and Myers, G.H.: Evidence against a vesico-renal reflex. J. Urol., 107:751, 1972.

Soloway, M.S., Burdick, J.F., Malmgren, R.A., and Myers, G.H.: Ileal conduit exfoliative cytology in the diagnosis of recurrent cancer. J. Urol., 107:835, 1972.

Futrell, J.W., Krueger, G.R., Morton, D.L., and Ketcham, A.S.: Carcinoma of sweat glands in adolescents. Amer. J. Surg., 123:594-597, 1972.

Soloway, M.S., Larsen, S., Johnston, G., and Myers, G.H.: Radioisotope renography in patients with an ileal conduit: The importance of the sitting position. J. Urol., 108:202, 1972.

Neel, H.B., Hammond, W.G., and Joseph, W.: Recurrent pulmonary embolism. Minn. Med., 55:15, 1972.

Catalona, W.J., Crowder, W.L., and Chretien, P.B.: Occurrence of hernia of Morgagni with filial cervical hernia: A hereditary defect of the cervical mesenchyme? Chest, 62:340-342, 1972.

Shimkin, P., Soloway, M.S., and Jaffee, R.: Metastatic melanoma in the gallbladder. Amer. J. Roentgen., 116:393-395, 1972.

Shimkin, P., and Soloway, M.S.: Blood borne metastases to the kidney: Investigation of three vascular tumors. Acta Radiologica, 12:387, 1972.

Myers, G.H., Jr., and MacPherson, B.R.: Histologically similar testicular neoplasms occurring in brothers. J. Urol., 108:757-759, 1972.

Soloway, M.S., Myers, G.H., Jr., Johnston, G., and Ketcham, A.S.: Renography in the evaluation of patients with ileal conduits. Surg. Gynec. Obstet., 135:521-524, 1972.

Soloway, M.S., Larsen, S.M., Myers, G.H., Jr., Johnston, G.S., and Ketcham, A.S.: Value of renography following radical pelvic cancer surgery. J. Surg. Oncol., 4:306-319, 1972.

Johnson, R., Harrah, J.D., and Ketcham, A.S.: Ill-advised biopsy. Amer. Surg., 38:357-363, 1972.

Birnholz, J.C.: Dermatoglyphics in congenital heart disease. Amer. J. Roent., 116:539-547, 1972.

Ketcham, A.S., Chretien, P.B., Hoyer, R.C., Harrah, J.D., Deckers, P.J., Sugarbaker, E.V., Taylor, P.T., and Rabson, A.S.: Occult metastases to the scalene lymph nodes in patients with clinically operable carcinoma of the cervix. Cancer, 31:180-183, 1973.

In Press:

Dellon, A.L., and Ketcham, A.S.: Surgical treatment of Stage I melanoma. Arch. Surg.

Geelhoed, G.W., and Ketcham, A.S.: Pelvic neoplasms causing pain during coitus. Med. Aspects of Human Sexuality.

Geelhoed, G.W., and Ketcham, A.S.: Pseudomonas meningitis complicating radical resection for radiocurrent cancer of the paranasal sinuses: Report of two patients successfully treated with intrathecal polymyxin. J. Surg. Oncol.

Geelhoed, G.W., Kane, M., Dale, D., and Wells, S.A.: Colon ulceration and perforation in cyclic neutropenia. J. Ped. Surg.

Geelhoed, G.W., and Myers, G.H., Jr.: Primary melanoma of the male urethra. J. Urol.

Ketcham, A.S., Chretien, P.B., Schour, L., Ommaya, A.K., and Van Buren, J.M.: Surgical treatment of patients with advanced cancer of the ethmoid sinuses. In Neoplasia of the Head and Neck (A Collection of papers presented at the 17th Annual Clinical Conference at the University of Texas, M.D. Anderson Hospital and Tumor Institute), Chicago, Ill., Year Book Medical Pub., Inc., 1973.

Pilch, B.Z., Kahn, C.R., Ketcham, A.S., and Henson, D.: Thyroid cancer after radioactive iodine diagnostic procedures in childhood. Pediatrics.

Millar, R.C., and Ketcham, A.S.: Tracheostomy obstruction secondary to a T-adapter. Anesthesiology.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3801

Title: Evaluation of Cancer Surgery as to its Effectiveness and Safety in Providing Definitive and Therapeutic Care of Cancer Patients

Principal Investigators: A.S. Ketcham, M.D., P.B. Chretien, M.D., and the entire Staff of the Surgery Branch, NCI

Lab/Branch: Surgery Branch

Goal: To increase the cure rates in patients with cancer of the cervix and head and neck region, melanoma and sarcomas by improved methods and diagnosis, new innovations in surgical treatment, and adjunctive therapy protocols.

Approach: Eligible cancer patients admitted to the Surgery Branch receive extensive diagnostic evaluation, including advanced techniques in the early stages of clinical evaluation. The treatment protocols utilized will determine the efficacy of approach that incorporate radical surgery with technical innovations designed to reduce morbidity and hasten rehabilitation. The contribution to treatment results of adjuvants to surgical therapy are evaluated in collaboration with various non-surgical clinical units of the NCI.

Progress: Aggressive surgical treatment of cancer of the cervix recurrent after definitive radiation treatment has yielded 5-year cure rates that justify this approach to our otherwise incurable disease. Analysis of treatment results with head and neck cancer have lead to the initiation adjunctive chemotherapy for those patients with poor prognosis after surgical therapy alone. Innovations in reconstruction techniques after head and neck cancer surgery have lead to more rapid and superior functional rehabilitation. In patients with melanoma, new approaches to regional lymph node dissection yield maximum cure rates with greatly reduced morbidity compared with conventional surgical techniques. To further increase these cure rates, new diagnostic protocols have been devised, and treatment regimens employing immunotherapy of melanoma have been formalized. The experimental evidence for control of sarcoma metastases with Coumadin have lead to a surgical treatment protocol employing adjunctive chemotherapy and Coumadin anticoagulation. More refined diagnosis of breast lesions and other accessible tumors have been achieved with concomitant reduction in morbidity and hospitalization time by the development of a technique of fine needle aspiration and immediate cytologic examination.

Significance to Cancer Research (NCP Objective 4, Approach 4): Surgical treatment remains the single effective modality for most malignancies. Measures to reduce the morbidity and increase the effectiveness of this modality by new techniques and by employment of adjunctive therapies offers improvement in salvage rates after cancer treatment with presently available methods.

Level of Effort: Professional Man-Years: 5

Serial No. NCI-3805
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Experimental Studies of Mechanisms of Metastases

Previous Serial Number: Same

Principal Investigator: A.S. Ketcham, M.D.

Other Investigators: P.B. Chretien, M.D., R.C. Millar, M.D.,
H.C. Hoover, M.D., G.B. Bulkley, M.D., A.M. Cohen, M.D.,
E.V. Sugarbaker, M.D., and Hilda Wexler

Cooperating Units: Laboratory of Pathology, NCI

Man Years:

Total: 3-1/2
Professional: 1
Other: 2-1/2

Project Description:

- Part I. Acetylsalicylic Acid Effect on Platelet Aggregation and Tumor Metastases
- Part II. The Effect of Combined Coumadin and Adriamycin Treatment on Tumor Metastases in Mice
- Part III. Serum-Mediated Facilitation of the Survival and Propagation of Circulating Tumor Cells
- Part IV. Altered Antigenicity of Metastases from an Antigenic Primary Tumor
- Part V. The Metastatic Potential of Experimental Pulmonary Metastases

Project Description:

- Part I. Acetylsalicylic Acid Effect on Platelet Aggregation and Tumor Metastases

Objectives:

It has been shown by Gasic, et al., that thrombocytopenia can cause a decreased number of pulmonary metastases in animals receiving an intravenous injection of

tumor cells. Preliminary evidence has also shown that platelet aggregation may be of importance in the lodgment and growth of tumor cell emboli in the vascular system. Acetylsalicylic acid (ASA) has been shown to paralyze the platelet's ability to aggregate. There have been some studies showing that ASA can decrease the number of pulmonary metastases in mice. Further work by Dr. Gasic, et al., has pointed out that some tumors have the ability to cause platelets to aggregate, while other murine tumors do not possess this ability. Using these findings he has been able to predict those animal tumor systems wherein ASA treatment would cause a decrease in number of metastases.

Methods Employed:

Experimental animals were given ASA in doses ranging from 15 mg/kg/24 hours to 250 mg/kg/24 hours, by adding it to their drinking water. Bleeding times were performed by making a uniform cut in the tail and measuring the time that bleeding ceased. The effect of ASA on platelet aggregation was determined by use of a chronolog aggregometer. The ability of different murine tumors (MCA-induced fibrosarcomas) to initiate platelet aggregation were tested. ASA effects on tumor metastases were examined by injecting methylcholanthrene-induced fibrosarcoma cells (approximately 1×10^5) into the footpad of mice. These tumors were allowed to grow to 1-1/2 to 2 cm in diameter, at which time the tumor-bearing extremity was amputated. All mice were then followed for an additional three weeks, at which time they were sacrificed, the lungs inflated with India ink, and the number of tumor metastases determined macroscopically. Mice treated with ASA were compared to controls receiving tap water.

Major Findings:

We have found that the bleeding time of mice is quite variable, although the animals treated with ASA in dosages of 100 mg/kg/24 hours do have a prolongation in their bleeding time as compared to controls. ASA-treated animals demonstrated a decrease in platelet aggregation when stimulated with 1 mcg of adrenalin. The results of ASA treatment in controlling metastases in one murine tumor system showed 74% incidence of metastases in the control group as compared to 57% in the ASA treated group. This particular MCA-induced tumor had very weak platelet aggregating ability. The effects of ASA on the incidence of metastases of a tumor with very active platelet aggregating ability are being studied at the present time.

Significance to Biomedical Research and Program of the Institute:

If various human tumors are shown to be strong stimulators of platelet aggregation, their metastatic potential may be altered by such agents as ASA.

Proposed Course:

Human tumors of various histologic types will be tested for any propensity to aggregate the host's platelets. A protocol for treating patients with ASA

will be developed. Multiple animal tumor systems will be studied comparing non-platelet aggregating and platelet aggregating tumors and the effects of ASA on their pattern of metastases.

Project Description:

Part II. The Effect of Combined Coumadin and Adriamycin Treatment on Tumor Metastases in Mice

Objectives:

There is considerable evidence in the literature suggesting that the blood coagulation system has an effect on tumor metastases. Hypercoagulative states have been shown to result in increased numbers of metastases as compared to hypocoagulative states in experimental animals receiving a bolus of tumor cells injected intravenously. There is evidence to show that tumor cells can produce a thromboplastin-like material, that fibrin deposited around tumor cells is related to growth and division of tumor cells, and that a fibrin coat around tumor cells may protect them from the effects of cytotoxic agents. The purpose of this project is to see if the combination of Coumadin anticoagulation and Adriamycin is superior to Adriamycin alone in decreasing pulmonary metastases.

Methods Employed:

Methylcholanthrene-induced fibrosarcoma cells were injected into the footpad of mice. The tumors were allowed to grow to sufficient size that metastasis to the lungs would occur. The primary tumor was then amputated. After three weeks the mice were sacrificed and the number of tumor metastases determined macroscopically by differential staining of lung and tumor tissue with an India ink solution. One-half of the mice received tap water throughout the experiment, the other half of the mice received sodium warfarin (1.7 mg/liter) added to their drinking water. The warfarin was started as soon as a palpable tumor was noted in the animal and continued until 14 days postamputation. Prothrombin times were done on mice receiving tap water and those receiving Coumadin in groups of 10 over the period of the experiment. On the second day following amputation, the tap water and Coumadin groups of mice were divided in half, each receiving either saline or Adriamycin in a dose of 1 mg/kg every three hours for a total of eight doses. They received the drug or saline intraperitoneally in a volume of 0.2 ml.

Major Findings:

The effects of warfarin anticoagulation alone upon this particular mouse tumor system has been shown to decrease the incidence of pulmonary metastases in these mice from 33% in the control animals receiving tap water to 4% in the animals receiving sodium warfarin. Effects of Adriamycin and Coumadin combined showed no significant improvement over anticoagulation or Adriamycin alone.

Significance to Biomedical Research and Program of the Institute:

There are several clinical studies suggesting that anticoagulation combined with chemotherapy is superior to chemotherapy alone. Very little data has been accumulated in animal tumor systems to substantiate these effects. It is hoped that further studies in the animal tumor systems may increase our knowledge of the role that the coagulation system plays in the initiation and growth of blood-borne metastases. These same mechanisms may also aid us in treating tumors with chemotherapeutic agents.

Proposed Course:

The methods as outlined are to be applied to several different tumor systems. Tumor metastases induced by intravenous injection of tumor cells will be used and the effects of Adriamycin and anticoagulation will be evaluated.

Project Description:Part III. Serum-Mediated Facilitation of the Survival and Propagation of Circulating Tumor CellsObjectives:

To determine the effect of serum from animals bearing immunogenic tumors on the production of pulmonary embolic tumors by circulating tumor cells shed from the tumor.

Methods Employed:

Pooled 2 ml blood aliquots were aspirated from the left hearts of normal C57Bl/6N mice and syngeneic mice bearing a fourth generation MCA-induced tumor and pulmonary metastases. The aliquots were centrifuged and the serum separated from the packed cells. Normal mice were inoculated I.V. with 1 ml mixtures of 0.5 ml of serum and 0.5 ml of packed cells. After 42 days, the animals were sacrificed and examined for lung tumors. The incidence of lung tumors in each group was correlated with the source of the serum and cells inoculated intravenously.

Major Findings:

A high incidence (17/14, 66%) of lung tumors was produced by the inoculation of animals with serum and cells from tumor-bearing hosts. By contrast, only 8/41 (20%) mice inoculated with a mixture of cells from tumor-bearing mice and normal serum had lung tumors and only 4/41 (10%) mice inoculated with serum from tumor-bearing mice and normal serum had lung tumors. These results show that the serum contained in the 1 ml aliquot of blood inoculated was necessary for the production of lung tumors by the circulating tumor cells in the blood inoculum.

Significance to Biomedical Research and Program of the Institute:

The results of this experiment show that the induction of tumor immunity in this animal tumor system facilitates the survival and propagation of circulating tumor cells and that this effect is serum mediated. Furthermore, it shows that the serum contained in 1 ml blood aliquots is sufficient to facilitate the survival of the circulating tumor cells in these aliquots. This in vivo effect of serum in facilitating the survival of circulating tumor cells appears to be analogous to the protective effect of immune serum on tumor cell monolayers exposed to specifically cytotoxic lymphocytes. This mounting evidence for the dominant effect of serum in facilitating the survival of tumor cells in the presence of immune cellular and humoral tumor-destructive mechanisms indicates that control of metastatic tumor production will be possible only after the development of methods that inhibit the effect of these serum factors. In addition, immunotherapy protocols must be carefully scrutinized for their effect on the production of these factors. Immunization with tumor antigen or non-specific stimulation of the immune system may augment the production of these serum factors and thus neutralize the effect of the tumor-destructive agents elicited by the therapy.

Proposed Course:

Fractionation of immune serum from animals bearing MCA tumors by physical and immunochemical techniques is in progress with the goal of defining the serum-mediated factor demonstrated in this experiment.

Project Description:

Part IV. Altered Antigenicity of Metastases from an Antigenic Primary Tumor

Objectives:

To test the hypothesis that antigenic differences exist between various metastases from a primary tumor and between these metastases and the primary tumor.

Methods Employed:

A fibrosarcoma was induced in syngeneic Fisher rats by injection of benzpyrene suspended in mineral oil. Immunogenicity was demonstrated in the third and fourth generations of the tumor. One rat was chosen for study, which had three large discrete metastases in the lungs. They were dissected free and designated respectively as M0, M1, and M2. Differences in the immunogenicity between these metastases and the primary tumor, designated P, were determined by the classical methods of tumor inoculation, amputation, and tumor challenge.

Major Findings:

M0 produced tumor in 27/30 controls versus 0/10 M0-immunized rats ($p < .01$). M2 produced tumor in 11/30 controls versus 0/10 M2-immunized rats ($p < .01$). P grew 21/30 controls versus 0/10 P-immunized rats ($p < .01$). These data show that M0, M2, and P lines are all significantly immunogenic. However, there was no significant inhibition of M0 or M2 tumor incidence in P-immunized rats, growing in 10/11 and 9/12, respectively, and similar to their growth in 27/30 and 11/30 controls, respectively. Mean latency periods of M0 and M2 in P-immunized rats were similar to those in control rats. These data indicate that the cell suspension derived from transplanted metastases grew uninhibited in rats immunized with the primary. Additional comparisons are of interest. M0 or M2 immunization significantly decreased the take of P compared to its growth in controls - 1/9 in M0-immunized and 4/11 in M2-immunized versus P growth in 21/30 controls ($p < .05$). Immunization with M0 did not significantly inhibit the take of M2 (5/10) compared to growth in 11/30 controls, and immunization with M2 did not significantly decrease the take of M0 (7/10) compared to growth in 27/30 controls. However, tumor latency periods were prolonged. These latter comparisons indicate that the metastases can be immunogenically dissimilar not only from the primary but also from each other and that some cross-reactivity may persist.

Significance to Biomedical Research and Program of the Institute:

These data offer considerable support for the concept that metastases may develop as a consequence of differential immunogenicity between the primary tumor and its metastases. Reasoning thus, tumor hosts may be unable to reject cells shed from the primary tumor because they are immunologically different from the primary. The consequences of this for eventual clinical application of immunotherapy are great in that most immunotherapy will be directed against metastatic disease. Hence, the differential immunogenicity of the metastatic tumor may have to be taken into account in developing modalities for immunotherapy.

Proposed Course:

This experimental model will be adopted for the study of differences in immunogenicity between primary tumors and metastases in human cancer patients.

Project Description:Part V. The Metastatic Potential of Experimental Pulmonary MetastasesObjectives:

To determine the effect of tumor-specific immunity on the propensity for tumor formation by circulating tumor cells shed from metastatic tumors.

Methods Employed:

Two tumor systems were used: An immunogenic MCA-induced tumor and a non-immunogenic S91 melanoma. C57Bl/6N mice were used with the first tumor and CDF1 mice with the second. The design was identical in both experiments. Mice with previously amputated primary tumor bearing legs and pulmonary metastases were sacrificed and their left heart blood aspirated. The blood was used to inoculate mice pairs intravenously (I.V.). These recipients were designated Group 2 mice. The pairs were divided into two groups, Group 2A and Group 2B. Each of these groups were further divided into two additional groups according to the time of sacrifice after inoculation of blood: Group 2A-1 was sacrificed 24 hours after inoculation, Group 2B-1 was sacrificed one week after inoculation, and Groups 2A-2 and 2B-2 were sacrificed six weeks after inoculation. When Group 2 mice were sacrificed, left heart blood was aspirated and inoculated into normal recipients, designated Group 3. These mice received the same subgroup designations as the Group 2 blood donors. When several mice in Group 3A-2 and 3B-2 died approximately six weeks after inoculation, all Group 3 mice were sacrificed.

Major Findings:

The results were similar with both animal tumor systems studied. In the C57Bl/6N mice inoculated with MCA-induced tumors, no lung tumors were found in Group 2 mice that were sacrificed one day and one week after I.V. inoculation of blood containing circulating tumor cells, nor were any found in Group 3 mice inoculated with blood aspirated from these Group 2 mice. In Group 2 animals sacrificed six weeks after inoculation, a high incidence of lung tumors was produced: 14/18 in Group 2A and 13/16 in Group 2B-2. Also, lung tumors were produced in Group 3 animals inoculated with blood from these mice: 5/10 in Group 3A-2 and 5/11 in Group 3B-2. In the CDF1 mice inoculated with S91 melanoma, Group 2 animals sacrificed on one day and one week after tumor inoculation had no lung tumors, neither did their respective Group 3 blood recipients. On the other hand, a total of 35/42 Group 2 mice had lung tumors when sacrificed six weeks after inoculation, and the blood from these animals produced lung tumors in 15/30 Group 3 recipients. These results showed that blood from mice with pulmonary metastatic tumors readily produced lung tumors when inoculated into normal mice. In Group 2 mice the possibility existed that the lung tumors produced may have generated from cells shed from the previously amputated primary tumor. However, the subsequent transfer of blood to other mice (Group 3) produced tumors only with blood from Group 2 mice that had large lung tumors, thus demonstrating that their origin is in circulating tumor cells shed from pulmonary tumors, not from the primary tumor. The experiment thus demonstrates that pulmonary metastases may shed cells that survive and produce new tumors, and since the results were the same with an immunogenic tumor as with a non-immunogenic tumor it demonstrates that cells from metastases of an immunogenic tumor enjoy the same facilitation in their propagation as cells from non-immunogenic tumors.

Significance to Biomedical Research and Program of the Institute:

These observations may have clinical significance to one of the most perplexing problems in clinical oncology - the metastatic potential of tumor metastases. Frequently, in a patient with a controlled primary tumor and a single metastasis deposit, a waiting period is elected to allow for the appearance of additional metastases. This course entails a theoretical danger of allowing the dissemination of cells from the metastases that produce tumors elsewhere. The data give no support to the hypothesis often advanced that immunity may destroy circulating tumor cells. Until data from human experiments show that this phenomenon does not occur in humans, the clinician must consider the experimental evidence which indicates that metastatic deposits shed cells that can colonize elsewhere in the tumor-immunized host.

Proposed Course:

Experiments now in progress are designed to define both the specific and non-specific serum factors that facilitate the survival and propagation of circulating tumor cells in this experimental model.

Publications:

Sugarbaker, E.V., and Cohen, A.M.: Altered antigenicity in spontaneous pulmonary metastases from an antigenic murine sarcoma. Surgery 1:155-161, 1972.

In Press:

Ketcham, A.S., Wexler, H., and Chretien, P.B.: The metastatic potential of experimental pulmonary metastases. J. Surg. Res.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3805

Title: Experimental Studies of Mechanisms of Metastases

Principal Investigator: A.S. Ketcham, M.D.

Section: Office of the Chief

Lab/Branch: Surgery Branch

Goal: The elucidation of factors which facilitate the circulation and secondary colonization of cells shed from a primary malignancy.

Approach: Studies are conducted in inbred mice and rats using chemically-induced immunogenic tumors and spontaneously arising tumors. The clinical evidence that serum factors in the tumor-immune host may protect rather than destroy circulating tumor cells has led to the development of experimental models that investigate the effect of humoral factors on the survival and propagation of circulating tumor cells. The role of immunoselection in the survival of immunogenic circulating tumor cells has been explored. The previous findings in this laboratory that Coumadin anticoagulation inhibits the colonization of circulating tumor cells have been expanded to the investigation of the effects of Coumadin combined with Adriamycin chemotherapy and the effects of acetylsalicylic acid in this animal-tumor system.

Progress: These investigations show that the survival and propagation of circulating tumor cells is facilitated by serum factors in tumor-immune and tumor-bearing hosts. Furthermore, these studies demonstrate the presence of both tumor-specific factors as well as non-specific factors that have this tumor-protective effect. Studies show that immunoselection may be important in determining the fate of circulating tumor cells. The inhibitory effect of Coumadin on the development of metastases has been extended to another animal tumor system, and a similar effect of acetylsalicylic acid has been demonstrated. Further augmentation of the anti-metastases effect of Coumadin was not obtained by the addition of Adriamycin chemotherapy.

Significance for Cancer Research (NCP Objective 1, Approach 4): Since a major cause of treatment failure in human malignancy is the development of fatal tumor metastases, these studies have direct relevance to the elucidation of factors determining the survival and propagation of metastasizing tumor cells and the development of approaches to the control of metastases formation in human cancer patients.

Level of Effort: Professional Man-Years: 1

1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Surgical Physiology and Metabolic Changes Associated with Extensive Cancer Surgery

Previous Serial No.: Same

Principal Investigators: R. M. Beazley, M.D. and R. C. Hoye, M.D.

Other Investigators: G. W. Geelhoed, M.D., S. H. Bennett, M.D., R. Aaron, M.D., G. C. Riggle, B.S., J. P. Grant, M.D., C. Gorschboth, B.S., R. J. Lewis, M.D., P. Re, M.D., W. K. Engel, M.D., and L. Schour, M.D.

Cooperating Units: Department of Anesthesiology, Blood Bank, Department of Nursing, Radiopharmaceutical Services, Biomedical Engineering Instrument Branch, Clinical Center, NIH.

Man Years:

Total:	7
Professional:	4-1/2
Others:	2-1/2

Project Description:

Part I. Application of Total Parenteral Nutrition to Postoperative Cancer Patients

Part II. Studies on the Etiology, Pathogenesis and Treatment of the Syndrome Post-traumatic Pulmonary Insufficiency

Part III. Fluid Volume and Albumin Kinetics

Part IV. Effects of Autotransfusion on Blood Elements

Part V. Surgical Stress and Coagulation

Part VI. Measurement of Hepatic Blood Flow in Primates

Part VII. Type II Muscle Fiber Atrophy and Malignant Disease

A. Incidence of Type II Muscle Fiber Atrophy in Patients with Malignant Disease

- B. Follow-up Study of Type II Muscle Atrophy in Treated Cancer Patients
- C. Animal Models of Type II Muscle Atrophy in Malignant Disease
- D. Type II Muscle Atrophy and Respiratory Function in Malignant Disease

Project Description:

Part I. Application of Total Parenteral Nutrition to Postoperative Cancer Patients

Objectives:

The importance of adequate nutrition in the promotion of wound healing and resistance to infection has long been known. The availability of hypertonic dextrose solutions with protein hydrolysates now provides a technique whereby adequate nutrition can be maintained in patients unable to tolerate an oral diet. A protocol was developed to standardize the application of total parenteral nutrition to postoperative patients and to study the metabolic effects on the stressed patient.

Methods Employed:

A registrar has been established to record insertion of subclavian catheters and complications of a non-infectious and infectious nature. Patients are screened prior to surgery for kidney and liver function and their metabolic status assessed by complete blood chemistries. During therapy patients are followed at weekly intervals with repeat blood studies and 24 hour urine collections to determine a daily metabolic balance.

Major Findings:

No complications from placement of 22 subclavian catheters have occurred in 15 patients. Cultures of catheter tips and blood cultures through the catheter at the time of removal revealed no primary catheter infections. Total parenteral nutrition has been given from 2 to 35 days with an average of 12.9 days. In all patients studied a positive nitrogen balance was obtained with gradual weight gain. No major electrolyte complications occurred as patients were followed closely and corrections made immediately. An inadequate number of patients have been followed to determine if there is a decrease in morbidity and hospital stay with total parenteral nutrition, but to date no increased morbidity has resulted. Three patients have received essential amino acids and hypertonic dextrose solutions for treatment in two of renal failure and in one of hepatic failure. In both cases of renal failure, blood urea nitrogen was found to decrease and stabilize and serum potassium returned to normal. In the patient with hepatic failure, blood ammonia levels decreased from 2-3 times normal and stabilized slightly above normal.

To ascertain if physical means could be used to prevent pulmonary injury resulting from aged blood perfusion, in seven additional baboons 21 day ACD-stored autologous blood was passed through a dacron-wool filter prior to perfusion through the left lower lobe of the primate lung perfusion model. The results were compared with similar perfusion of fresh (24 hour stored ACD blood) and stored (21 day aged ACD blood).

To determine whether pharmacologic means could be used to block or prevent the development of pulmonary injury resulting from stored blood perfusion, two groups of seven baboons each underwent lung perfusion with stored autologous bloods after pretreatment with steroids in seven animals and with Trasylol in another seven animals. In each of the previously listed experiments, pulmonary arterial perfusion pressures (P_{pa}), endobronchial pressure (P_b), and arteriovenous gas tensions (by mass spectrometry) were continuously monitored during perfusion while pulmonary venous pressure was maintained at zero. Lung biopsies were done before and at the conclusion of perfusion for gross and microscopic estimation of pulmonary morphologic changes.

Major Findings:

For those primate lungs which were perfused with blood reconstituted from stored plasma and fresh cells, a 33% rise in pulmonary vascular resistance was seen over that which occurred with fresh blood perfusion and there was twice the decrease in effective compliance. Arteriovenous pO_2 gradient decreased 28% from that seen with fresh blood perfusion.

Perfusion with stored blood uniformly caused pulmonary functional and morphologic degeneration. None of the changes seen with stored blood perfusion were seen with fresh blood perfusion.

For blood that had been dacron wool-filtered, gross and microscopic evidence of pulmonary edema developed in 4/7 of the perfused lungs and pulmonary vascular resistance increased 16% over that seen with fresh blood perfusion although effective compliance values were the same as that seen in fresh blood perfusion.

Animals pretreated with Methyl Prednisolone were partially protected from the effects of aged blood, and to a greater degree than that seen with Trasylol, which had previously been reported to be effective in blocking pulmonary changes seen with septic blood whereas steroid had been ineffective.

It was concluded that, within the limits of our model, pulmonary injury can result from perfusion with bank blood which can be mediated to some extent by the plasma fraction of stored blood and cannot be eliminated by dacron wool-filtration. Some of the effect of stored blood perfusion on pulmonary function can be prevented by pretreatment with high dose corticosteroid, whereas Trasylol appears to have afforded minimum protection.

Significance to Biomedical Research and Program of the Institute:

The findings from this series of experiments have enlightened at least one aspect of post-traumatic pulmonary insufficiency, insofar as it can be mediated

Significance to Biomedical Research and Program of the Institute:

It is felt when properly monitored that total parenteral nutrition is a safe procedure in postoperative cancer patients and offers an early return to positive nitrogen balance and subsequent benefits of early wound healing and more rapid recovery. The utilization of essential amino acids and hypertonic dextrose solutions provide a useful mode of therapy for the hypercatabolic patient with borderline to failing renal function in the treatment of elevated blood urea nitrogen and potassium. The use of essential amino acids in hepatic failure will need more research before any general conclusions can be drawn.

Proposed Course:

Continuation of clinical studies and development of animal experimental model to further evaluate essential amino acids and liver function.

Project Description:

Part II. Studies on the Etiology, Pathogenesis and Treatment of the Syndrome Post-traumatic Pulmonary Insufficiency

Objectives:

Many patients undergoing major cancer surgery or suffering severe injury succumb to progressive pulmonary failure. A single common etiologic factor identified and studied in the Surgery Branch of the National Cancer Institute has been the administration of massive quantities of homologous blood transfusion. Massive transfusion is of unquestioned, life-saving necessity for circulatory volume restoration, but some evidence exists that transfusion with multiple units of stored bank blood might be deleterious to lung function due to cellular debris and other unknown agents in stored blood. Using an in situ pulmonary lobe perfusion system in the dog and primate lung, we have developed an experimental model to study the effects of infusion of stored blood on lung function, have attempted to isolate and identify factors in stored blood that cause lung dysfunction, and have attempted physical and pharmacologic methods to block and prevent the pulmonary injury that results from stored blood perfusion.

Methods Employed:

The method and model for the study of pulmonary function with aged blood perfusion have been described in a publication from the Surgery Branch this year. The methods described produced results showing uniform production of a "shock-lung"-like syndrome with bank blood perfusion. Following the demonstration of this effect, an attempt was made to isolate the fraction of the stored blood responsible for this damage. Autologous primate blood was stored for 21 days in ACD solution, passed through a standard filter, and then separated into cellular and plasma components. Fresh cells were suspended in aged plasma, and aged cells were suspended in fresh plasma and these reconstituted solutions were employed in the in situ primate lung perfusion model.

To ascertain if physical means could be used to prevent pulmonary injury resulting from aged blood perfusion, in seven additional baboons 21 day ACD-stored autologous blood was passed through a dacron-wool filter prior to perfusion through the left lower lobe of the primate lung perfusion model. The results were compared with similar perfusion of fresh (24 hour stored ACD blood) and stored (21 day aged ACD blood).

To determine whether pharmacologic means could be used to block or prevent the development of pulmonary injury resulting from stored blood perfusion, two groups of seven baboons each underwent lung perfusion with stored autologous blood after pretreatment with steroids in seven animals and with Trasylol in another seven animals. In each of the previously listed experiments, pulmonary arterial perfusion pressures (P_a), endobronchial pressure (P_b), and arteriovenous gas tensions (by mass spectrometry) were continuously monitored during perfusion while pulmonary venous pressure was maintained at zero. Lung biopsies were done before and at the conclusion of perfusion for gross and microscopic estimation of pulmonary morphologic changes.

Major Findings:

For those primate lungs which were perfused with blood reconstituted from stored plasma and fresh cells, a 33% rise in pulmonary vascular resistance was seen over that which occurred with fresh blood perfusion and there was twice the decrease in effective compliance. Arteriovenous pO_2 gradient decreased 28% from that seen with fresh blood perfusion.

Perfusion with stored blood uniformly caused pulmonary functional and morphologic degeneration. None of the changes seen with stored blood perfusion were seen with fresh blood perfusion.

For blood that had been dacron wool-filtered, gross and microscopic evidence of pulmonary edema developed in 4/7 of the perfused lungs and pulmonary vascular resistance increased 16% over that seen with fresh blood perfusion although effective compliance values were the same as that seen in fresh blood perfusion.

Animals pretreated with Methyl Prednisolone were partially protected from the effects of aged blood, and to a greater degree than that seen with Trasylol, which had previously been reported to be effective in blocking pulmonary changes seen with septic blood whereas steroid had been ineffective.

It was concluded that, within the limits of our model, pulmonary injury can result from perfusion with bank blood which can be mediated to some extent by the plasma fraction of stored blood and cannot be eliminated by dacron wool-filtration. Some of the effect of stored blood perfusion on pulmonary function can be prevented by pretreatment with high dose corticosteroid, whereas Trasylol appears to have afforded minimum protection.

Significance to Biomedical Research and Program of the Institute:

The findings from this series of experiments have enlightened at least one aspect of post-traumatic pulmonary insufficiency, insofar as it can be mediated

by stored blood. The Surgery Branch performs many major cancer operations which require massive transfusions every year, and it has been important for us to minimize the postoperative pulmonary complications of these patients. We have demonstrated the role that stored blood can play in the production of pulmonary injury and have attempted to further isolate and identify these injurious factors and develop a method of prevention. It appears that there is some toxic element in stored blood plasma and that separation of aged blood cells or filtration of aggregate particles in blood do not completely eliminate the toxic effect to the lung of bank blood perfusion. It appears that pretreatment with steroids may affect some benefit in blocking some deleterious effects to the lung from stored blood perfusion.

Proposed Course:

Progressive clinical application of these experimental findings will follow after the results from our experimental model have been fully analyzed and discussed.

Project Description:

Part III. Fluid Volume and Albumin Kinetics

Objectives:

To determine exact fluid and electrolyte arrangements occurring with extensive abdominal surgery.

Methods Employed:

Total red blood cell volume, plasma volume and extracellular fluid volume were determined by isotopic dilution techniques. These measurements were done pre-operatively immediately after surgery and during intervals in the postoperative recovery period. Albumin kinetics were monitored and measurements of total exchangeable body albumin pools were made.

Major Findings:

With more sophisticated techniques it was found that some patients, despite normal parameters, came to surgery with volume deficits as high as 38% from normal. A considerable amount of operative blood loss was found to be hidden in the operative drapes and was not generally accounted for. Operative plasma volumes were noted to decrease by 29%. Based on these findings, an increased infusion of albumin in relatively large quantities as indicated postoperatively.

Significance to Biomedical Research and Program of the Institute:

Metabolic changes following extensive cancer surgery have not previously been thoroughly investigated. To date, the quantitative studies being carried out in our laboratory in patients undergoing extensive cancer surgery have indicated a multiplicity of these deficits may occur. Increased knowledge obtained in

these studies in the management of postoperative patients will be dramatically changed in the near future. Morbidity and mortality should decrease significantly with institution of new treatment regimes.

Proposed Course:

Examination of this research program should lead to more precise determination of ideal replacement fluids and optimum rate of replacement during and after surgery.

Project Description:

Part IV. Effects of Autotransfusion on Blood Elements

Objectives:

To determine 1) feasibility and 2) effects of autotransfusion on blood elements.

Methods Employed:

With the aid of the Bio-Engineering Department a new type blood suction device for anticoagulation of autogenous blood was developed. This device not only allowed blood to be recovered from the operative site, but it also provided for anticoagulation, storage and restoration of coagulative properties prior to reinfusion. Using this device in described techniques, canine autotransfused blood was evaluated. In another project, blood obtained at the time of cancer surgery was compared to bank blood.

Major Findings:

In autotransfused canine blood there was noted to be a decreased hematocrit and a rise in plasma hemoglobin. There was also a significant decrease in platelet elements. No effect could be demonstrated on the clotting studies. Red blood cell survival was found to be normal. Moderately severe hemolysis was found in allotransfused blood obtained at the time of surgery. Microaggregations of small particles were found both in banked and autotransfused blood. In contrast to the acidotic bank blood pH of the autotransfused blood was 7.72.

Significance to Biomedical Research and Program of the Institute:

Perfection of an autotransfusion system will have direct human surgical application. It will enable more extensive surgery to be done without resorting to dependence upon normal banked blood.

Proposed Course:

Continued investigations in laboratory animals and clinical use of autotransfusion will be pursued to define the indications for the use of this alternative to blood transfusion.

Project Description:

Part V. Surgical Stress and Coagulation

Objectives:

To determine the relationship of surgical stress and coagulation on the development of postoperative venous thrombosis, interaction of the coagulation mechanisms and kinin generation in the production of the postoperative inflammatory response.

Methods Employed:

Normal coagulation techniques were utilized to monitor patients preoperatively, operatively, and postoperatively. In addition, platelet template bleeding times, glass bead adhesion, and platelet factor assays were utilized. Pre-kallikrein and kininogen levels were also monitored.

Major Findings:

There were two periods of hyperactive platelet function, namely the second and tenth days. Demonstration of activation of Hageman factor at the time of trauma was noted. Activation of the kinin precursor system was also observed to begin intraoperatively. These findings are collectively consistent with the clinical observation of appearance of postoperative inflammation and coagulation changes.

Significance to Biomedical Research and Program of the Institute:

These studies serve to provide a laboratory basis for a clinical understanding of the relationship between surgical stress, blood coagulation, and inflammation. Correlation with the clinical development of tumor metastases may show a metastases-potentiating effect of the changes in blood coagulation that occur with surgical stress.

Proposed Course:

Future investigations will attempt to determine the feasibility of control of blood coagulation during surgical stress to reduce vascular thrombosis and minimize any effect on tumor metastases.

Project Description:

Part VI. The Measurement of Hepatic Blood Flow in Primates

Objectives:

The goal of this project was to devise a surgical model for chronically studying hepatic blood flow in primates. The wash-out of radioactive xenon from the hepatic vascular bed was to be compared with the standard method of BSP excretion for determining total hepatic blood flow. The contribution to total hepatic

blood flow by the hepatic artery and portal vein was to be determined. The effect of gastrointestinal hormones on blood flow to the liver was to be studied

Methods Employed:

Rhesus monkeys and adult baboons were prepared in the following manner: A splenectomy was performed. Small silastic catheters were inserted into the hepatic artery and portal vein. These were brought through the abdominal wall and occluded with a blunt needle having a rubber diaphragm over the end. This blunt needle was sutured to fascia. Needles were placed through the skin into the blunt needles for injection of xenon or other study materials. The hepatic artery was isolated on the celiac access by ligating the left gastric, the pancreaticoduodenal, the cystic, and the right gastric arteries. An electromagnetic flow meter was placed around the hepatic artery. A pneumatic occluder was also placed downstream to the electromagnetic flow meter. A third silastic catheter was placed in the hepatic vein.

Total hepatic blood flow was determined by BSP excretion during constant infusion. Hepatic blood flow was also determined from the radioactive xenon washout curves over the liver. The hepatic artery and portal vein sites were used for injection of xenon 133 and the flows from each compared to that calculated by BSP excretion. Hepatic artery blood flow was measured by the electromagnetic flow meter. Pitocin and glucagon were injected into the animal at different times and hepatic blood flow as measured by xenon-133 washout and the electromagnetic flowmeter were compared.

Major Findings:

The surgical model for studying hepatic blood flow has been successful in allowing us to study primates multiple times over a 10-14 day period. Total hepatic blood flow as measured by BSP excretion (523 ± 155 ml/min) compares very favorably with hepatic blood flow as measured by xenon-133 washout curves when the xenon was injected via the portal vein (509 ± 158 ml/min). Pitocin had the effect of decreasing total hepatic blood flow and glucagon increasing total hepatic blood flow, both to a significant amount. In both instances, the hepatic artery seemed to recover more quickly from the effect of these two drugs as compared to the portal vein blood flow.

Significance to Biomedical Research and Program of the Institute:

A method for evaluating total hepatic blood flow in patients would be very helpful in evaluating patients with liver disease prior to undergoing surgery. An understanding of the flow patterns within the liver may also be of use in designing chemotherapy for liver metastatic disease.

Proposed Course:

This project will be continued with the hope of developing a non-traumatic method for estimating total hepatic blood flow in humans. Other gastrointestinal hormones or active peptide fractions that have effect upon the gastrointestinal system will also be studied to determine their effects upon total hepatic blood flow.

Project Description:Part VII. Muscle Fiber Atrophy and Malignant DiseaseA. Incidence of Type II Muscle Fiber Atrophy in Patients with Malignant DiseaseObjectives:

To determine the incidence of histochemically determined type II muscle fiber atrophy in patients with malignant disease, without weight loss or clinical or neuromuscular abnormality.

Methods Employed:

(This study was carried on primarily under the aegis of the Medical Neurology Branch, NINDS.) Preoperative patients with seven different tumor types, inpatients at the Clinical Center, Surgery Branch, National Cancer Institute, were studied. Following a negative neurological evaluation and negative evaluation for metastatic disease, open muscle biopsy and open muscle electromyography were performed in the operating room. Electrical muscle behavior was correlated with histochemistry from the same muscle sample. The routine battery of histochemical muscle tests employed by the Medical Neurology Branch, NINDS, was employed in the study of muscle specimens.

Major Findings:

No electromyographic abnormality, aside from failure to develop full interference pattern in some patients, was noted. All patients studied demonstrated some degree of type II histochemical fiber atrophy, independent of the type or stage of their cancer. This finding has not previously been reported in asymptomatic patients with localized malignancy.

Significance to Biomedical Research and Program of the Institute:

Remote effects of malignant disease on the neuromuscular system have been reported in a number of tumors. The presence of histochemical type II muscle fiber atrophy in asymptomatic patients regardless of tumor type was a surprising finding. This result suggests that remote metabolic effects of cancer may be present at an earlier stage than appreciated heretofore. The study of this phenomenon might be of aid in the diagnosis of otherwise occult malignancy, and the follow-up of patients treated for malignant disease.

Proposed Course:

Further investigations of histochemical structure of skeletal muscle in patients with malignant disease are proposed to determine the incidence of type II fiber atrophy with various tumor types and stages.

B. Follow-up Study of Type II Muscle Atrophy in Treated Cancer Patients

Objectives:

To determine the effect of adequate surgical treatment on the type II muscle fiber atrophy observed preoperatively in patients with malignant disease.

Methods Employed:

Patients studied preoperatively and found to have type II muscle fiber atrophy were rebiopsied under local anesthesia approximately six months following definitive surgery. The degree of type II fiber atrophy present was correlated with the degree of preoperative atrophy. This also correlated with the patient's clinical status (presence or absence of recurrence). An attempt was made using television scanning to quantitate the degree of type II muscle fiber atrophy present preoperatively and six months following surgical treatment.

Major Findings:

In those patients studied postoperatively, type II muscle fiber atrophy did not progress or was slightly diminished in those patients free of disease. One patient found to have a clinical recurrence had no regression of type II atrophy. Quantitative studies of all patients available for rebiopsy are in progress.

Significance to Biomedical Research and Program of the Institute:

If, on the basis of this study, it is determined that the type II atrophy present in malignant disease is reversible with cure of the malignancy, the techniques of muscle biopsy and histochemical staining can be employed as an indicator of clinical status and allow the early detection of recurrences at a time when they can be effectively treated. The natural history of type II fiber atrophy associated with malignant disease will also be elucidated in a clinical population.

Proposed Course:

All patients from the original group studied preoperatively that are available for rebiopsy will be studied. Qualitative and quantitative evaluation of the degree of type II muscle fiber atrophy relative to the degree present preoperatively will be determined. Histochemical findings will be correlated with clinical status.

C. Animal Models

Objectives:

To provide an animal model for the study of type II muscle fiber atrophy in association with malignant disease.

Methods Employed:

Two host systems were studied. Methylcholanthrene-induced sarcomas were implanted in the hind quarter of Sprague-Dawley rats and spontaneous hepatomas in the hind quarter of strain 2 guinea pigs. At regular intervals following the implantation of a known tumor dose, animals were sacrificed and distant muscles examined for the presence of histochemical type II muscle fiber atrophy. The degree of atrophy was assessed qualitatively and quantitatively. The presence of atrophy was correlated with weight gain or loss and absolute body weight as well as tumor weight.

Major Findings:

In spite of large tumor masses, muscles from the Sprague-Dawley rats did not demonstrate a well-defined type II muscle fiber atrophy. In preliminary experiments, histochemical fiber type definition was superior as was the determination of type II fiber atrophy when the guinea pig was employed.

Significance to Biomedical Research and Program of the Institute:

An animal model for studying the relationship of histochemical type II muscle fiber atrophy and malignant disease is of value in determining the natural history of the phenomenon and to delineate whether the type II fiber atrophy is a specific remote effect of cancer or part of a generalized metabolic imbalance produced by malignant disease. Elucidation of the potential for reversal of type II atrophy following cure or removal of the lesion could provide leads to the usefulness of serial biopsies as a clinical tool in evaluating the treatment of cancer patients.

Proposed Course:

The natural history of the development of type II muscle fiber atrophy will be studied in the guinea pig system. Reversal of type II fiber atrophy following amputation (cure) of the primary tumor will be studied. The development of type II fiber atrophy as it relates to weight gain or loss and the generalized metabolic state of the animal will be studied. Further studies could include biochemical and physiologic investigations of the underlying mechanism of production of type II muscle fiber atrophy in association with malignant disease.

D. Type II Muscle Atrophy and Respiratory Function in Malignant Disease

Objectives:

To assess the role of type II muscle atrophy in clinical respiratory problems in cancer patients.

Methods Employed:

At the time of definitive surgery, depending on the nature of the operation, biopsies are taken from the primary and accessory respiratory musculature of cancer patients. These biopsies are processed by the usual battery of histochemical stains and examined for type II muscle fiber atrophy. Clinical course, pulmonary function tests, and postoperative respiratory problems are correlated with the degree of type II muscle fiber atrophy present in the primary and accessory respiratory musculature.

Major Findings:

Type II atrophy, sure to be present in the proximal skeletal musculature of asymptomatic cancer patients, was found to be present in the accessory muscles of respiration. Clinical correlation with respiratory complications following surgery, neuromuscular problems involving respiration, and pulmonary function tests are being carried out.

Significance to Biomedical Research and Program of the Institute:

Patients undergoing surgery for cancer have a high proportion of postoperative complications. Many of the most serious complications are respiratory. This study may help to define a possible neuromuscular component to respiratory problems in patients with malignant disease.

Proposed Course:

The data obtained from operative muscle specimens will be correlated with pulmonary function tests in the clinical course of the patients. As other studies elucidating possible treatments for carcinomatous muscle atrophy begin to yield results, it may be possible to reduce the incidence of postoperative respiratory problems in patients with malignant disease by treatment of their underlying neuromuscular lesion.

Publications:

Hoye, R.C., Bennett, S.H., Geelhoed, G.W., and Gorschboth, C.: Fluid volume and albumin kinetics occurring with major surgery. J.A.M.A. 222:1255-1261, 1972.

Bennett, S.H., Hoye, R.C., and Riggle, G.C.: Intra-operative autotransfusion. Preliminary report of a new blood suction device for anticoagulation of autologous blood. Amer. J. Surg. 123:257, March, 1972

Bennett, S.H., Geelhoed, G.W., Gralnick, H.R., and Hoye, R.C.: Effects of autotransfusion on blood elements. Amer. J. Surg. 125:273-279, March, 1973.

Bennett, S.H., Geelhoed, G.W., Aaron, R.C., Solis, R.T., and Hoye, R.C.: Pulmonary injury resulting from perfusion with stored bank blood in baboon and dog. J. Surg. Res. 13:6 295-306, 1972.

Riggle, R.C., Bennett, S.H. and Hoyer, R.C.: Regional anticoagulation for intraoperative reinfusion of autologous blood. Proc. Congress on Engineering in Medicine and Biology, pp. 196, 1972.

Bennett, S.H., Geelhoed, G.W., Terrill, R.E., and Hoyer, R.C.: Pulmonary effects of autotransfused blood retrieved from the pleural cavity in an in situ perfusion model. Amer. J. Surg., in press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3806

Title: Surgical Physiology and Metabolic Changes Associated With Extensive Cancer Surgery

Principal Investigator: R. M. Beazley, M.D. and R. C. Hoye, M.D.

Section: Office of the Chief

Lab/Branch: Surgery Branch

Goal: To evaluate both established and newer surgical physiological concepts as they apply to the diagnosis, treatment, and management of the cancer patient.

Approach: Utilizing both laboratory and clinical techniques, we are evaluating:

- 1) Application of total parenteral nutrition to postoperative cancer patients.
- 2) The etiology, pathogenesis and treatment of the syndrome of post-traumatic pulmonary insufficiency.
- 3) Fluid volume and albumin kinetics in surgical patients.
- 4) The effects of autotransfusion on blood elements.
- 5) Surgical stress and coagulation
- 6) Techniques to measure hepatic blood flow in primates
- 7) Type II muscle fiber atrophy and malignant disease.

Progress: Studies have shown a serum factor involved in post-traumatic pulmonary insufficiency. Parameters for administration of volume and albumin in extensive surgery have been isolated. The effects of autotransfusion on blood elements has been evaluated in the lab and clinically. A relationship between surgical stress and changes in blood coagulation has been demonstrated. Progress has been made towards development of a non-invasive hepatic blood flow measuring technique. Muscle fiber atrophy has been demonstrated to occur in cancer patients.

Significance for Cancer Research (NCP Objective 6 Approach 5):

To cure as many patients as possible and to maintain maximum control of the cancerous process in patients not cured. To develop and utilize methodologies of immunology, chemotherapy, surgery, and radiation alone and in combination and the cure and palliation of surgery.

Level of Effort: Professional Man-Years: 4-1/2

Serial No. NCI-3816
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NCI
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cryobiology

Previous Serial Number: Same

Principal Investigator: R. M. Beazley, M.D.

Other Investigators: A. S. Ketcham, M.D., H. B. Neel, III, M.D., and
D. Bagley, M.D.

Cooperating Units: Laboratory of Pathology, National Cancer Institute, and
Biomedical Engineering and Instrumentation Branch, National
Institutes of Health

Man Years:

Total: 1-1/2
Professional: 1/2
Others: 1

Project Description:

Part I. Effect of Cryodestruction on the Primate Facial Nerve

Part II. Effect of Cryodestruction on the Canine Recurrent Laryngeal Nerve

Part III. Alterations of Blood Sugar and Plasma Insulin After Cryosurgery of Functioning Eyelet Cell Tumor

Part IV. Experimental Evaluation of In Situ Oncocide for Primary Tumor Therapy

Project Description:

Part I. Effect of Cryodestruction on the Primate Facial Nerve

Objectives:

To determine the effect on structure and function of primate facial nerve which has been subjected to cryodestruction.

Methods Employed:

Serial No. NCI-3816

The Linde CE2, PR-5 probe was modified so that it could be mounted and held in an operating microscope stand. Utilizing microminiature thermocouples and a high speed 6-channel recorder, cryolesions were made on surgically exposed primate facial nerve.

Major Findings:

Complete ipsilateral facial palsy has been produced in all animals. Gradual return of function was monitored by serial motion picture recordings for the next six to eight weeks. Complete and normal nerve function resumed in all animals within eight weeks.

Significance to Biomedical Research and Program of the Institute:

The ability of the facial nerve to regain function after a segment of the nerve has been destroyed by cryodestruction has direct application in human tumor surgery. This may enable the surgeon to ablate inoperable tumors with cryosurgery while saving structure and function of important nerves in the immediate tumor area.

Proposed Course:

Future studies will be expanded to standardize the degree and extent of injury to determine maximally tolerable temperature depression of nerves that is compatible with functional recovery.

Project Description:

Part II. Effect of Cryodestruction of the Canine Recurrent Laryngeal Nerve

Objectives:

To determine the effect on structure and function of the canine recurrent laryngeal nerve which has been subjected to cryodestruction.

Methods Employed:

The Linde CE2, PR-5 probe was modified so that it could be mounted and held in an operating microscope stand. Utilizing microminiature thermocouples and a high speed 6-channel recorder cryolesions were made on the surgically exposed canine recurrent laryngeal nerve.

Major Findings:

Complete ipsilateral recurrent nerve palsy, with resultant vocal cord paralysis was noted in all animals thus treated. The return of function was monitored by repeated direct laryngoscopies over the following six to eight weeks. 90% of animals thus treated regained normal cord function within eight weeks.

Significance to Biomedical Research and Program of the Institute:

The ability of recurrent laryngeal nerve to return to function after undergoing cryodestruction may have widespread application in oncological surgery. This will enable the surgeon to ablate tumor tissue which may encroach on the nerve, and at the same time, save structure and function of the nerve and its innervated tissue.

Proposed Course:

Studies in progress will define tolerance of recurrent nerve to temperature depression and repetitive freezing.

Project Description:

Part III. Alterations of Blood Sugar and Plasma Insulin After Cryosurgery of Functioning Eyelet Cell Tumor

Objectives:

To ascertain parameters involved in cryosurgery of functioning endocrine tumors.

Methods Employed:

Islet cell tumors were implanted in the thigh muscle of Golden Syrian hamsters. After the tumors had reached 10-15 mm in size they were destroyed by cryosurgery. Blood sugar levels and serum insulin levels were monitored before, during, and after tumor inoculation and cryosurgery.

Major Findings:

In animals in which tumors were destroyed the depressed mean blood sugar levels returned to normal levels as did serial plasma insulin levels.

Significance to Biomedical Research and Program of the Institute:

This project demonstrates that a functioning endocrine tumor can be completely destroyed by cryosurgery. Previous studies from this laboratory have demonstrated the feasibility of cryodestruction of pancreatic tissue with minimal systemic effect on the animal. This technique may have direct application to the treatment of functioning pancreatic tumors. Furthermore, it has relevance to the utilization of new methodologies and surgical approaches to the cure and palliation of patients with these malignancies.

Proposed Course:

These experimental studies will continue with the goal of clinical trials in cancer patients.

Project Description:

Part IV. Experimental Evaluation of In Situ Oncocide for Primary Tumor Therapy: Comparison of Tumor Specific Immunity After Complete Excision, Cryonecrosis and Ligation

Objectives:

Test the hypothesis that in situ tumor cell necrosis, in addition to being a feasible means of curing primary tumors, increases host response against the tumor immunological mechanisms. A typical mammary adenocarcinoma and a methyl-cholanthrene induced sarcoma were utilized in mice. After the tumors had reached approximately 1 cm in diameter they were treated in the following ways: One group was completely surgically excised, one group was frozen using standardized cryosurgical technique, and the third group was treated by infarction of the tumor. Those animals which subsequently demonstrated complete cure were challenged five weeks later with varying dilutions of the same tumor cell suspension.

Major Findings:

In both the sarcoma group and in the breast carcinoma group, higher tumor challenge doses were needed to induce recurrent growth in those animals frozen as compared to those animals which underwent surgical excision. The immunological effect of freezing was greater than noted following ligation.

Significance to Biomedical Research and Program of the Institute:

The fact that in situ tumor necrosis produces greater immunity than surgical excision provides an experimental basis in immunology for the clinical results reported after cryosurgical destruction of various neoplasms. It also suggests that further investigations of in situ oncocide for treatment of surgically unresectable primary and metastatic tumors warranted for potential immunological effects.

Proposed Course:

Expanded investigations of the effects of freezing on host reactions to tumors in experimental animals are in progress. These will be oriented toward possible clinical applications in patients with inoperable tumors.

Publications:

Neel, H.B., III, Ketcham, A.S. and Hammond, W.G.: Experimental evaluation of in situ oncocide for primary tumor therapy. Laryngoscope pp. 376-386, March, 1973.

Neel, H.B., III, Myers, R.S., Ketcham, A.S., Hammond, W.G.: Alterations of blood sugar and plasma insulin after cryosurgery of functioning eyelet cell tumor. J. Surg. Oncol. 4:511-519, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3816

Title: Cryobiology

Principal Investigator: R. M. Beazley, M.D.

Section: Office of the Chief

Lab/Branch: Surgery Branch

Goal: To observe the pathophysiological changes which occur in both normal and neoplastic tissue as a result of cryodestruction.

Approach: Efforts along this line have been directed at:

1. The effect on structure and function of cryodestruction of normal facial and recurrent laryngeal nerves.

2. Comparing tumor specific immunity after a) freezing; b) conventional surgery; and c) ligation of neoplastic tissue.

Progress: Function of normal facial and recurrent laryngeal nerves is restored after intense freezing. Progress in cryodestruction in mice tumor systems evokes a greater amount of tumor-specific immunity than either conventional surgery or ligation.

Significance for Cancer Research (NCP Objective 6, Approach 1)

Level of Effort: Professional Man-Years: 1/2

Serial No. NCI-3817
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Subcellular Mediation of Immune Responses to Tumor Antigens

Previous Serial Number: Same

Principal Investigator: Y.H. Pilch, M.D.

Other Investigators: P.J. Deckers, M.D., B.Z. Pilch, M.D., and R.B. Hume

Cooperating Units: None

Man Years:

Total:	3
Professional:	1
Other:	2

Project Description:

Part I. Transfer of Tumor-Specific Immunity with RNA

Part II. The Effect of Fetal Antigens on the Growth of a Chemically-Induced Bladder Cancer

Part III. Immune Response of Tumor-Bearing Animals to Non-Tumor-Related Antigens

Project Description:

Part I. Transfer of Tumor-Specific Immunity with RNA

Objectives:

To isolate preparations rich in ribonucleic acid from cells and tissues which process antigens, such as lymph nodes and spleens of the animals immunized with tumors and to study the immunoreactive properties of these RNA preparations in mediating immune responses to tumor antigens. To utilize these preparations for immunotherapeutic purposes in experimental animal models.

Methods Employed:

RNA is prepared by phenol extraction of lymph nodes and spleens or peritoneal macrophages of experimental animals following immunization in vivo or in vitro

with tumor cells or following immunization of animals by temporary tumor growth. RNA is determined by the orcinol reaction, DNA by the diphenylamine reaction, and protein by the Lowery method. RNA is characterized by its sedimentation characteristics upon ultracentrifugation on sucrose density gradients. The tumor-host systems employed have been benzpyrene-induced sarcomas in inbred mice, methylcholanthrene-induced sarcomas in inbred mice, benzpyrene-induced sarcomas in inbred rats, and methylcholanthrene-induced sarcomas in inbred guinea pigs. Cellular immune responses were determined in vitro by a modification of the immune cytotoxicity assay of McKhann and in vivo by the ability of the immunized animal to demonstrate a significant reduction in the incidence of tumor development.

Major Findings:

RNA was extracted from the lymph nodes and spleens of guinea pigs previously immunized with benzpyrene-induced C₃H/He mouse sarcoma (BP4). Normal C₃H spleen cells were incubated with this RNA and injected into C₃H mice which were then challenged with 10⁴ viable BP4 cells. Marked decrease in the incidence of tumor growth resulted. RNA from guinea pigs immunized with normal C₃H tissue was ineffective. Treatment of the active RNA preparations with ribonuclease removed activity while deoxyribonuclease or pronase treatment did not. Specificity of this response for the immunizing tumor was demonstrated. In another system, RNA from the nodes and spleens of guinea pigs immunized with the Fisher 344 rat sarcoma (BP-1R) was mixed with dextran sulfate, a potent inhibitor of ribonuclease, and administered to normal Fisher rats, which were then challenged with 10⁴ BP-1R cells. Significant reduction in the incidence of tumor development was noted. Dextran sulfate alone or with non-specific guinea pig RNA was ineffective.

In a syngeneic system designed to conclusively establish RNA mediation of immunity to tumor-specific transplantation antigens, normal Fisher spleen cells were incubated with RNA from the spleens of Fisher rats immunized with BP-1R and given IV to Fisher rats which then received 3.5 x 10⁴ BP-1R cells. Inhibition to tumor growth resulted. In another syngeneic system, in vitro, normal strain 2 guinea pig spleen cells were incubated with RNA extracted from the spleens of strain 2 guinea pigs immunized with a methylcholanthrene-induced Strain 2 guinea pig sarcoma (MCA-A). Acellular areas of immune cytotoxicity of MCA-A cells were produced when RNA-incubated spleen cells were applied to MCA-A monolayers, but produced no cytotoxicity of normal strain 2 lung epithelial cells. Again, ribonuclease treatment abrogated the response, and specificity for the immunizing tumor was demonstrated.

Significance to Biomedical Research and Program of the Institute:

This work represents a model of yet another modality for the immunotherapy of cancer which, it is hoped, may have certain peculiar advantages. Some of these advantages are (1) it would obviate the necessity for the administration of heterologous or homologous antisera, or leukocytes. Administration of such sera or cells can sensitize to leukocyte isoantigens and may result in toxicity

or secondary syndromes. The implications of these studies for the immunotherapy of human cancer are as follows. Since heterologous RNA has been shown to be effective and non-toxic, animals other than human beings could be immunized with tumor tissue and immune RNA extracted from the lymphoid tissues. Alternatively, antigen processing cells such as peritoneal macrophages or lymph node cells from such animals could be exposed, in vitro, to human tumor tissue in tissue culture, and immune RNA could then be extracted. Such RNA might be administered to patients by direct injection by mixing the RNA with dextran sulfate. Dextran sulfate is available for clinical use in humans in clinical grades of purity. An alternative method will be to collect immunocompetent cells such as peripheral blood lymphocytes from patients by methods such as leukaphoresis on the NCI cell separator. These cells could then be incubated with the "immune" RNA and then returned to the patient from which they were obtained only a short time previously. Such a model for immunotherapy would offer several theoretical advantages. First, since antigen processing is bypassed, some anergic patients might be benefitted if the anergy were due to defects in antigen processing and recognition, and not due to a lack of immunocompetence of effector cells, i.e., lymphocytes. Second, this sort of immunotherapy would obviate the necessity of administering autologous or homologous tumor cells to cancer patients; (2) no adjuvant is required; (3) this model does not require the administration to patients of homologous or heterologous immunocompetent cells. Such cells contain leukocyte antigens to which the patient can be sensitized. In addition, a danger of graft versus host reaction exists. Moreover, immune elimination of such passively transferred immunocompetent cells might make prolonged immunotherapy ineffective. In our model, immune RNA would be injected directly or incubated with the patients' own autologous lymphocytes. The medicolegal problem of the source of donor leukocytes for immunotherapy is eliminated since leukocytes would not have to be obtained from other cancer patients or normal volunteers and no cross-immunization with tumor tissue would be required of other cancer patients or normal volunteers.

Proposed Course:

Efforts are now underway to isolate and characterize the active RNA in these preparations. There is evidence in the literature to suggest that such preparations may contain immunoreactive RNA-antigen complexes. An attempt will also be made to measure the mediation of immune cytolysis of human tumor cells, in vitro, by human lymphocytes pre-incubated with "immune" RNA prepared from guinea pigs immunized to human tumors. Experiments are also planned to treat established, progressively growing tumors in rodents with immune RNA mixed with dextran sulfate and with RNA-incubated spleen cells in an attempt to cause slowing of growth or regression of such established tumors.

Project Description:

Part II. The Effect of Fetal Antigens on the Growth of a Chemically-Induced Bladder Cancer

Objectives:

The purpose of this experiment was to determine the effect of immunization with irradiated mid-gestation syngeneic fetal tissue upon the subsequent growth of a chemically-induced murine bladder cancer. The work was stimulated by reports of others that immunization with fetal tissue could protect against subsequent challenge with a tumor in syngeneic hamster and guinea pig systems, thus offering evidence for cross-reactivity between fetal and tumor antigens.

Methods Employed:

The tumor used was a bladder tumor which had originally arisen in a C₃H/HeN mouse fed a diet containing the carcinogen N-[4-(4-Nitro-2-furyl)-2-thiazolyl]formamide. This tumor has been shown to be both transplantable and immunogenic. Both male and virgin female C₃H/HeN mice were immunized by four or five weekly injections of mid-gestation (10-14 days) syngeneic irradiated (5000 R) fetal cells. The animals received one million viable cells intraperitoneally and one million cells subcutaneously at each immunization. One to two weeks following the last immunization, all animals were challenged with the mouse bladder tumor injected intramuscularly in the right leg. Control groups included animals injected with irradiated syngeneic adult liver, kidney, and spleen cells, and untreated animals.

Major Findings:

Various doses of tumor cells were utilized, and in no case were there significantly fewer tumors in the fetal-immune group as opposed to the control groups; in fact, there has been a trend of increased tumor incidence in the fetal-immune group, although this difference has as yet not been shown to be statistically significant. Thus, we were unable to show protection by the prior exposure to fetal tissue against this chemically-induced bladder cancer.

Significance to Biomedical Research and Program of the Institute:

This experiment suggests that perhaps fetal antigens cross-reactive with tumor-specific transplantation antigens may not be universally present in all tumor systems or, if present, may not be detectable by in vivo tumor transplantation techniques. Thus, although the detection of fetal or embryonic antigens may be of diagnostic or prognostic significance in human cancer, much more work needs to be done to assess the possible therapeutic role of fetal antigens in human cancer.

Proposed Course:

Another group of male mice is currently under investigation using a still different dose of tumor, and it will be noted whether or not immunization with fetal antigens in this system may indeed significantly increase the tumor incidence. If this is found to be the case, then perhaps the fetal tissue may elicit a so-called blocking antibody which enhances rather than inhibits tumor growth.

Project Description:Part III. Immune Response of Tumor-Bearing Animals to Non-Tumor-Related AntigensObjectives:

The purpose of this study is to compare the immunologic response of tumor-bearing and non-tumor-bearing syngeneic laboratory animals to non-tumor-related antigens such as tuberculin and sheep red blood cells. Previous work by others has shown that tumor-bearing animals may have a depressed immunologic responsiveness to their own tumor, and other work has shown that some carcinogenic agents such as leukemia viruses and some chemical carcinogens may be themselves immunosuppressive. This study undertook to assess whether or not the presence of a tumor induced by transplantation of tumor cells rather than by carcinogenic agent per se was sufficient to reduce the immunologic responsiveness of these animals.

Methods Employed:

Balb/c, C₃H/HeN, and C₅₇-Bl female mice were used in this study. Groups of these mice were injected with syngenic tumors, a chemically-induced bladder tumor in the C₃H mice, a methylcholanthrene-induced sarcoma in the C₅₇Bl mice, and a spontaneously arising kidney tumor in the Balb/c mice. Age-matched controls were uninoculated. When the tumors arose, all animals were injected intraperitoneally with $1-2 \times 10^6$ sheep red blood cells. Animals were bled five and 14 days following red cell inoculation, and anti-sheep cell titers were measured by hemagglutination. In other experiments, strain 2 guinea pigs were inoculated with a syngeneic methylcholanthrene-induced sarcoma. Control guinea pigs were not inoculated. When tumors arose, animals were immunized with a complete adjuvant containing the H37RA strain of Mycobacterium tuberculosis. All guinea pigs were also inoculated intraperitoneally with sheep red cells. Two weeks later, blood was collected and guinea pigs were skin tested with tuberculin, and biopsies of the skin reactions taken.

Major Findings:

All results have not been fully collected and evaluated at the time of this report, since the experiment is still in progress.

Significance to Biomedical Research and Program of the Institute:

It is hoped that this study will help to explain whether the presence of a tumor per se is sufficient for immunodepression of the host or whether immune depression may rather have a role in genesis of the tumor. This may help to elucidate the relationship between cancer and immunity.

Proposed Course:

After analysis of the results of this experiment, an attempt will be made to

correlate the level of immune depression of the tumor-bearing host on the rate of growth of the malignancy, and if partial tumor removal results in recovery of immune competence.

Publications:

Deckers, P.J., and Pilch, Y.H.: Mediation of immunity to tumor-specific transplantation antigens by RNA: Inhibition of isograft growth in rats. Cancer Res. 32:839-846, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3817

Title: The Subcellular Mediation of Immune Responses to Tumor Antigens

Principal Investigators: Y.H. Pilch, M.D.

Section: Office of the Chief

Lab/Branch: Surgery Branch

Goal: To determine in murine animal-tumor systems the specificity of tumor immunity transferred by RNA, the relation of tumor antigens to fetal antigens, and the effect of tumor growth on non-tumor immunity.

Approach: A method for extracting RNA from spleen tissue was developed for study of the ability of RNA from guinea pig tumor-bearing hosts to confer specific immunity to murine tumor-bearing hosts. Cross-reactivity of tumor antigens and fetal antigens was explored using a chemically-induced bladder tumor. Alteration in immune reactivity of tumor-bearing murine hosts was determined by quantitating the response to non-tumor antigens.

Progress: RNA extracted from spleens of guinea pigs bearing benzpyrene-induced sarcomas conferred murine resistance to the growth of the same tumors when transplanted into Fisher rats. Appropriate controls showed that the effect of the RNA was specific for the neoplastic tissues. Experiments with a murine bladder carcinoma induced by an oral carcinogen show a trend towards the production of tolerance to tumor inocula by prior injection of mid-gestation fetal tissue. Evaluation of the effect on humoral antibody production to sheep red blood cells of chemical carcinogen-induced tumors in murine hosts is in progress.

Significance to Cancer Research (NCP Objective 4, Approach 4): These experiments have direct relevance to attempts at control of cancer in humans. The successful inhibition of experimental tumor growth by transfer of RNA in a heterologous system offers an approach for human immunotherapy. The results that RNA extracted from lymphoid tissue of non-human host previously inoculated with human cancer may have a therapeutic effect when inoculated in human cancer patients. Monitoring evidence for cross-reactivity or identity between fetal and tumor antigens suggest the use of fetal tissue in immunoprophylaxis and immunotherapy. The correlation between non-specific immunity and tumor behavior in human cancer hosts gives importance to the quantitation of non-tumor immunity and its relation to tumor induction and growth.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-3818
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Malignancy Associated Cellular and Serum Abnormalities

Previous Serial Number: Same

Principal Investigators: P.B. Chretien, M.D., W.F. Sample, M.D., and
W.J. Catalona, M.D.

Other Investigator: B.F. Cullen, M.D.

Cooperating Units: Anesthesiology Department, Clinical Center, NIH

Man Years:

Total:	4
Professional:	2
Other:	2

Project Description:

- Part I. In Vitro Lymphocyte Reactivity and Prognosis in Cancer Patients
- Part II. Correlation of Tumor Histologic Type and Clinical Stage with Cellular and Serum Abnormalities in Cancer Patients
- Part III. Quantitative Dinitrochlorobenzene (DNCE) Reactivity in a Normal Population
- Part IV. Quantitative DNCE Sensitivity in Cancer Patients
- Part V. Comparison of In Vivo and In Vitro Assays of Immunologic Reactivity in Cancer Patients
- Part VI. Serum Ribonuclease Levels in Cancer Patients
- Part VII. Peripheral Blood Macrophage Activity in Cancer Patients
- Part VIII. Cellular Immunity in Cured Cancer Patients
- Part IX. Correlations of Lymphocyte Reactivity and Tumor Histology with Genetic Profiles in Cancer Patients

Project Description:Part I. In Vitro Lymphocyte Reactivity and Prognosis in Cancer PatientsObjectives:

To determine the relationship of in vitro lymphocyte reactivity to phytohemagglutinin in patients with non-lymphoid malignancies and prognosis after definitive surgical treatment.

Methods Employed:

The in vitro lymphocyte reactivity was determined in 94 patients who had a variety of non-lymphoid malignancies. In each, the tumor was considered clinically operable and, after determination of the lymphocyte reactivity, definitive surgical removal was attempted. The lymphocytes of 120 volunteers in good health and in the same age range as the cancer patients provided control data for the scoring of the lymphocyte reactivity of the cancer patients. Individuals with a history of malignancy, acute or chronic illness, or who were taking any medication were excluded. In vitro lymphocyte reactivity was determined in autologous serum. Individual leukocyte cultures consisted of 2×10^6 mononuclear cells in a 4 ml mixture of 80% RPMI 1640 and 20% autologous serum. Phytohemagglutinin P (PHA) was added at five different concentrations ranging from 3.1 to 50 μ g of PHA/protein per ml of culture. Triplicate cultures with each dose of PHA and controls without PHA were prepared. After 54 hours incubation, tritiated thymidine was added, and at 72 hours the cultures were terminated. The nuclear protein was precipitated with trichloroacetic acid and the precipitate processed for scintillation counting. The radioactivity of each culture was determined in a Model 303 Packard Tri-Carb scintillation counter. The results were corrected for quench and expressed in disintegrations per minute (DPM).

Major Findings:

To derive an age-correlated range of normal lymphocyte reactivity, the 120 control subjects were divided into five groups of approximately equal age intervals and the mean reactivity ± 1 standard deviation (SD) determined for each group. The lymphocyte reactivities of the control population have a normal distribution, i.e., 84 patients (70%) had lymphocyte reactivity within ± 1 SD of the mean, and 18 patients (15%) each had reactivity greater than and less than 1 SD of the mean. The highest lymphocyte reactivity occurred in the youngest individual studied. From a peak in the second decade, reactivity declined over the next four decades, then was relatively constant through the last decade studied.

Three years after completion of the determination of lymphocyte reactivity of the cancer patient, the medical records of the patients were reviewed. Follow-up was complete on all patients. The patients were classified either

as (1) clinically cured of tumor at the time of the review (34 patients), (2) as inoperable (29 patients), or (3) as having recurrent tumor - either at the site of operation or in the form of distant metastases - after tumor resection that was considered complete (31 patients). The lymphocyte reactivity of each patient was arbitrarily defined in relation to the reactivity of the control population. The reactivity within ± 1 SD of the mean age-correlated control reactivity was classified as normal, reactivity greater than 1 SD of the mean control reactivity was classified as high, and reactivity less than 1 SD of the mean control reactivity was classified as low. The distribution of the lymphocyte reactivity of the cancer patients was significantly lower than that of the controls. The difference is due primarily to the percent of cancer patients with low lymphocyte reactivity (45% compared with 15% of controls). Significantly fewer cured cancer patients, however, had low lymphocyte reactivity than did inoperable patients or patients with recurrent tumor. There was a significantly greater percent of cured patients with inoperable or recurrent tumors (7%).

Significance to Biomedical Research and Program of the Institute:

The relationship shown in this study of in vitro lymphocyte reactivity to extent of tumor growth and prognosis after surgical treatment shows that this test assays a clinically significant aspect of the host-tumor relationship. The studies which demonstrate a correlation between the delayed hypersensitivity response to dinitrochlorobenzene and prognosis in cancer patients and those which show that the DH response to DNCB parallels in vitro lymphocyte reactivity when considered together suggest that in vitro lymphocyte reactivity provides similar insight into the prognosis of cancer patients as DH to DNCB. These two tests combined, therefore, may have increased value as a means of predicting the course of cancer patients who receive definitive treatment of their malignancies.

Proposed Course:

Continued improvement in this assay system to provide more quantitation of in vitro lymphocyte reactivity and extension of the assay to include patients clinically cured of tumor, and continued assay of patients studied preoperatively will further determine the relationship of in vitro reactivity to susceptibility to cancer, effect of cancer treatment, and the feasibility of adjunctive therapy which operates through alteration of immunologic responsiveness.

Project Description:

Part II. Correlation of Tumor Histologic Type and Clinical Stage with Cellular and Serum Abnormalities in Cancer Patients

Objectives:

To quantitate abnormalities of in vitro lymphocyte reactivity and serum effects on lymphocyte reactivity in cancer patients and determine the incidences

of these abnormalities in patients with varied histologic types of tumors and the relationship of tumor stage.

Methods Employed:

Preoperative patients with non-lymphoid tumors of a variety of histologic types were studied. Control data was obtained from healthy volunteers in the same age range as the cancer patients. Volunteers with a history of chronic disease, medication ingestion, recent illness, or history of malignant tumor were excluded. An AB pool, prepared from healthy volunteers, was used as the constant reference source for both cellular and serum reactivity. Purified specimens of mononuclear cells were obtained from the cancer patients and the controls by the exposure of whole blood to carbonyl iron and separation of the cells that phagocytize the iron particles. Cultures containing 5×10^5 mononuclear cells/ml of 90% RPMI 1640 and 10% AB pooled serum were prepared. Phytohemagglutinin P (PHA) was used in five concentrations to determine the peak response to PHA. Cultures with each dose of PHA and controls without PHA were prepared in triplicate. After a culture interval of 24 hours, tritiated thymidine was added for a three hour labelling period, after which the cultures were terminated. The cultures were processed for scintillation counting and the radioactivity expressed in disintegrations per minute (DPM).

Major Findings:

The cellular reactivity of the cancer patients was arbitrarily defined in terms of the response of normal volunteers: It was normal if it was within 1 SD of the mean for the response of volunteers of the same age group, high if it exceeded the mean by more than 1 SD, and low if it was less than 1 SD of the mean. As a group, significantly more patients with cancer had low reactivity than controls (30% versus 16%). When lymphocyte reactivity was correlated with histology, the highest incidence of low reactivity was seen in patients with squamous carcinomas (45%) and sarcomas (41%). Patients with melanomas and adenocarcinomas as a group had the same lymphocyte reactivity as the control patients. When the patients were divided into three groups according to the stage of tumor growth, there was no correlation between tumor stage and lymphocyte reactivity. Using the AB serum pool as a constant reference, the effect of sera from cancer patients on the reactivity of normal lymphocytes showed that serum from patients with squamous carcinomas and sarcomas significantly inhibited the reactivity of normal lymphocytes. Serum from patients with melanomas and adenocarcinomas did not display this abnormality. Serum and cellular abnormalities coexisted in patients with sarcomas but occurred independently in patients with squamous carcinomas.

Significance to Biomedical Research and Program of the Institute:

A major finding in this study is the association of a greater incidence of low lymphocyte reactivity in patients with tumors of certain histologic types. This information is invaluable in planning immunotherapy regimens that attempt

to correct immunological defects. Second, the relationship of low lymphocyte reactivity to development of tumor may be pertinent to the epidemiology and control of cancer. If the low lymphocyte reactivity preceded the tumor in these patients, assays of this type may be useful as a means of screening for cancer-prone individuals and detection of carcinogenic environmental conditions. If low lymphocyte reactivity is the consequence of cancer, whether reversion to higher reactivity occurs with clinical cure should be determined. If it does, assay of lymphocyte reactivity may be a sensitive means of monitoring effects of tumor treatment. The results of this study indicate that only patients with certain histologic types of tumors can be monitored in this manner. The demonstrated relationship of serum suppression factors to tumor histologic type also provide a new, rational basis for the design of immunotherapy treatment protocols based on tumor histology. Failure to demonstrate serum suppressants of lymphocyte reactivity in patients with melanomas and adenocarcinomas may indicate that tumor vaccine treatment for these patients is less likely to have the undesirable effect of suppressing immunity by invoking the production of these serum factors than in patients with epidermoid carcinomas and sarcomas.

Proposed Course:

Studies in progress will determine the nature of the serum inhibitory factor demonstrated and those factors which elicit its production. Control of the action of this factor is of obvious importance in immunologic approach to eradication of malignancy. The effect of immunological adjuvants on the alteration of the suppressed lymphocyte responsiveness is also being studied.

Project Description:

Part III. Quantitative Dinitrochlorobenzene (DNCB) Reactivity in a Normal Population

Objectives:

To derive age-correlated data concerning normal DNCB reactivity determined with a method that quantitates allergy to DNCB.

Methods Employed:

Because of the limitations of previous methods of determining delayed hypersensitivity, which score patients as either anergic or allergic, a scheme for quantitating allergic response to DNCB was devised. A 2000 µg dose of DNCB was applied to the upper arm simultaneously with the application of a 50 µg dose to the volar forearm of normal individuals. The subjects were examined daily for a minimum of 21 days. A spontaneous flare reaction at both the 2000 µg and 50 µg sites by the fourteenth day is graded 4+. A spontaneous flare at the 2000 µg site alone is graded 3+. In the absence of a spontaneous flare at the 2000 µg site, a challenge dose of 50 µg is applied on the fourteenth day. An unequivocal DCH response after 48 hours is graded 2+.

Equivocal reactions are biopsied. If these show a mononuclear cell infiltrate, the reaction is graded 1+. Two months after completion of the above studies, additional subjects were selected for the study of the threshold of DNCB necessary to elicit an anamnestic response. Correlations were made between the reactivity observed with the primary response and the dose of DNCB required to elicit an anamnestic response.

Major Findings:

Of the 143 subjects tested, 139 (97%) were sensitized to DNCB by the criteria used. All but one of the sensitized subjects exhibited either a 3+ or 4+ reaction. The mean interval between sensitization and a spontaneous flare reaction was 9-10 days for the 2000 µg and 11-12 days for the 50 µg dose. There was no correlation between age and the positive response to DNCB. Four subjects were anergic and, although they were all over 40 years of age, the ages were equally distributed through the last four age decades studied. Subjects who had primary 4+ reactions developed an anamnestic response to DNCB challenge with a mean dose of 5 µg of DNCB. Subjects who had 3+ reactions responded to a mean dose of 25 µg of DNCB. Although a trend toward higher reactivity was observed in patients with a history of allergy, no statistically significant difference could be established. Similarly, no correlation could be demonstrated between reactivity to DNCB and race, family history of cancer, the use of alcohol, or the use of tobacco.

Significance to Biomedical Research and Program of the Institute:

While sensitive techniques have been developed for the quantitation of humoral antibody responses, methods of measuring cell-mediated immunity are crude by comparison. The elicitation of delayed cutaneous hypersensitivity reactions to intradermally injected antigens has often been employed to measure cell-mediated immunity in vivo. However, these antigens yield limited information concerning cellular immune responsiveness since most are complex biologic extracts which cite a combination of both humoral and cellular immune responses. Furthermore, they are widely employed to elicit anamnestic responses which rely upon previous exposure to respond to a newly-encountered antigen. The limitations of intradermal DCH tests are obviated by the use of DNCB. The agent is an allergen of definite known constitution; therefore, sensitization and challenge can be accurately controlled in terms of time and dose, and experimental conditions are exactly reproducible. Humoral antibodies are not involved in DCH reactions to DNCB; thus, it is a specific test for cell-mediated immunity that assesses the current ability of a subject to manifest DCH to newly-encountered antigen. This method of quantitating DCH to DNCB, therefore, provides the first method of quantitating in vivo immunologic responsiveness and will be invaluable in assessing the immunologic status of cancer patients before and after treatment and in quantitating the effects of therapy regimens on immunologic responsiveness.

Proposed Course:

This method of quantitating DCH to DNCB was used to measure the immunologic responsiveness of all patients admitted to the Surgery Branch for definitive cancer therapy. Studies of patients cured of cancer are also in progress to determine the relationship of the abnormalities demonstrated preoperatively to prognosis.

Project Description:Part IV. Quantitative DNCB Sensitivity in Cancer PatientsObjectives:

To quantitate in vivo immunologic responsiveness in patients with non-lymphoid tumors using an assay method that quantitates delayed hypersensitivity (DH) to DNCB.

Methods Employed:

All patients with non-lymphoid tumors admitted to the Surgery Branch for definitive treatment of their malignancies undergo preoperative assay of DH to DNCB. The method employed incorporates the spontaneous flare reaction which occurs to the residual DNCB remaining in the skin after sensitization. A spontaneous flare at the 2000 μ g site and the 50 μ g sites by fourteen days after sensitization is graded 4+. A spontaneous flare at the 2000 μ g site only is considered 3+, and a response elicited only after challenge with 50 μ g is considered 2+. Reactions that require biopsy to demonstrate are graded 1+. The reactivities of the cancer patients were correlated with tumor histologic type and clinical stage.

Major Findings:

Only 41% of cancer patients developed spontaneous flare reactions to DNCB, an incidence that is significantly less than in controls (96.5%). An additional 29% required challenge to elicit DCH to DNCB, and 30% were anergic. When the patients were grouped according to histologic type of tumor present, all groups had significant abnormal reactivity. The incidence of anergy was highest in patients with squamous carcinomas (43%), less in patients with melanomas (25%) and adenocarcinomas (26%), and not significantly different from controls in patients with sarcomas (12%). Of patients with squamous carcinomas, 28% had impaired positive reactivity which, when combined with the incidence of anergy, made them the most abnormal group. The impaired positive reactivity in patients with melanomas (44%) and adenocarcinomas (26%) further magnifies the abnormality of their expected reactivity. In patients with sarcomas, among whom the proportion of anergy was not significantly different from controls, the 25% incidence of impaired positive reactivity rendered the distribution of their reactivities significantly abnormal when compared with controls. When the patients were divided into two groups

according to the extent of tumor determined preoperatively and at operation, and those with localized tumors and those with metastatic tumors, there was no statistically significant difference in the distribution of reactivities in the two groups. When considered by tumor histologic type, patients with sarcomas showed a relationship between impaired reactivity to DNCB and extent of tumor present.

Significance to Biomedical Research and Program of the Institute:

The most important contribution of this assay method is the impaired positive reactivity (2+ and 1+) revealed in allergic cancer patients. These levels of impaired reactivity occurred in a high incidence among patients with tumors of all histologic types studied, thus significantly increasing the proportion in each group with abnormal reactivity. That the incidence of abnormal reactivities was highest in patients with squamous cancer, significantly less in patients with melanoma and adenocarcinoma, and least in those with sarcoma, yet with the exception of sarcoma was not related to clinical extent of tumor, indicates differing host-tumor relationship exists which is determined by the histologic type of tumor present. Therefore, in investigations of cellular immune responses of cancer patients, it is essential to consider tumor histology. This study of cancer patients and the previous study of normal controls demonstrates the method of quantitating DH to DNCB distinguishes at least three levels of reactivity (4+ and 3+, 2+ and 1+, and anergy) which appear to be biologically meaningful.

Proposed Course:

The subsequent clinical course of the patients entering this study will eventually show the relationship of the levels of DH to DNCB demonstrated and prognosis of cancer patients who receive definitive treatment of their tumors. The assay method is also incorporated into an immunotherapy protocol which selects patients on the basis of immunocompetence. The assay will also be used to monitor the effects of the treatment on immune responsiveness.

Project Description:

Part V. Comparison of In Vivo and In Vitro Assays of Immunologic Reactivity in Cancer Patients

Objectives:

To compare in vitro cellular immunity as assayed by lymphocyte response to phytohemagglutinin (PHA) and in vivo immunologic responsiveness assayed by quantitated contact sensitivity to dinitrochlorobenzene (DNCB).

Methods Employed:

Patients admitted to the Surgery Branch who had clinically operable malignancies were selected for study. All patients were in good general health

and had no chronic or acute disease, and were taking no medications. No patient had undergone general anesthesia, radiotherapy, chemotherapy, or steroid therapy for at least three months prior to testing. DNCB contact sensitization was determined using the quantitative scheme developed by this unit. This scheme measures four distinct levels of positive responsiveness as outlined elsewhere in this section. In vitro lymphocyte responsiveness to phytohemagglutinin in short-term tissue culture was determined by quantitation of tritiated thymidine incorporation. The cells were cultured in homologous AB serum to obviate the effect of serum inhibitors of lymphocyte reactivity.

Major Findings:

The age-correlated lymphocyte reactivity of 185 patients was compared with DNCB reactivity. This comparison revealed a correlation coefficient of .86 which is significant at the 95% confidence level. When levels of DNCB reactivity are expressed in terms of mean lymphocyte reactivity, at least three distinct levels of lymphocyte reactivity are quantitated in terms of DNCB reactivity. This comparison gives further validity to previous data which showed that the method of assessing DNCB reactivity provides a semiquantitative measure of sensitivity to DNCB.

Significance to Biomedical Research and Program of the Institute:

This study demonstrates that lymphocyte in vitro reactivity to PHA parallels DH to DNCB, but each assay contributes unique data. Lymphocyte reactivity to PHA further quantitates cellular reactivity at the extreme of DH to DNCB and also reveals serum inhibitors of cellular reactivity as described elsewhere in this section. DH to DNCB tests in vivo cellular mechanisms only but does so in the presence of serum inhibitors. Therefore, the utilization of both assays provides complementary data concerning the immunologic reactivity of cancer patients. Because of the mounting evidence that the immunologic reactivity of cancer patients determines the results of treatment and that the alterations in immunologic responsiveness induced by chemotherapy and immunotherapy correlate with the effects of such therapy, it is increasingly important that the immunologic reactivity of cancer patients be accurately determined. This study demonstrates that these two assays are effective in this goal.

Proposed Course:

With the validity of both of these assays established as methods that quantitate immunologic reactivity, both will be used to quantitate reactivity prior to the institution of definitive therapy and to monitor the effects of therapies, especially chemotherapy and immunotherapy.

Project Description:

Part VI. Serum Ribonuclease Levels in Cancer Patients

Objectives:

To determine the significance of elevated serum ribonuclease levels in cancer patients. To relate this abnormality with tumor histologic type and stage.

Methods Employed:

Serum alkaline ribonuclease levels were measured by spectrophotometric quantitation of soluble products of RNA digestion by serum ribonucleases. Optimum activity occurred at pH 7.8. AB pooled serum specimens from a single source was used as a constant reference point. Age-correlated criteria of normal ribonuclease levels were determined through a study of serum from over 200 normal subjects. The sera of over 90 cancer patients with a variety of histologic types and stages of malignancies were similarly assayed. All cancer patients were in otherwise good health. Patients with a history of chronic disease or who were taking medications were excluded.

Major Findings:

A significant correlation between serum ribonuclease levels and tumor histologic type was found. Patients with melanomas and sarcomas had ribonuclease levels which as a group did not differ from that of normal controls. Patients with squamous carcinomas and adenocarcinomas, however, had significantly elevated ribonuclease levels. There was no correlation between tumor stage and ribonuclease levels in patients with these latter two types of tumors.

Significance to Biomedical Research and Program of the Institute:

This study demonstrates that patients with squamous carcinomas and adenocarcinomas have significantly elevated alkaline ribonuclease levels. Further study of this phenomenon through assay of the same patients after definitive treatment of their tumors and assay of patients clinically cured of cancer will determine the significance of these findings. If these studies show a return to normal levels after tumor removal of the ribonuclease measured, the use for this assay as a tumor "marker" would appear feasible. Patients with elevated ribonucleases that persist after tumor removal would therefore suggest continued presence of tumor. In such patients, this assay would allow the earlier institution of adjuvant therapy for subclinical residual disease.

Proposed Course:

Continued study of the serum of these patients after definitive tumor treatment and assay of patients clinically cured of tumor are in progress to determine the usefulness of this assay as an indicator of clinically occult tumor.

Project Description:Part VII. Peripheral Blood Macrophage Activity in Cancer Patients

Objectives:

To quantitate peripheral blood macrophage phagocytosis and enzymatic processes in cancer patients.

Methods Employed:

A technique for isolation of blood phagocytes (neutrophils and monocytes) was developed. Phagocytic activity was quantitated by the determination of latex polystyrene particle phagocytosis. Enzymatic digestive mechanisms were quantitated by reduction of p-nitro tetrazolium blue (NBT) in response to latex particle ingestion. Cancer patients who were in otherwise good general health were studied. None had other chronic diseases and all were afebrile and had normal peripheral blood cell counts. Normal controls in the same age group were studied to establish normal control values for these assays.

Major Findings:

In contrast to the findings with lymphocyte reactivity, macrophage function in 60 normal volunteers showed no relationship with age. In over 100 preoperative patients, both latex phagocytosis and NBT reduction were suppressed compared with controls. When tumor histology was considered, minor variations were seen, but no group was statistically different from any other. A study of cured cancer patients now in progress shows, in general, normal macrophage function, but study of larger numbers of patients will be necessary for statistically significant comparisons.

Significance to Biomedical Research and Program of the Institute:

This investigation reveals hitherto unappreciated abnormalities in cancer patients. The mounting evidence that macrophage processing of tumor antigen is a necessary prerequisite for immunologic response indicates that defects in phagocytosis may account for impaired host resistance to cancer. The studies of this aspect of immune mechanism should, therefore, provide significant insight into the host-tumor relationship. In addition, therapy regimens should be devised that correct this abnormality.

Proposed Course:

Continued study of all patients prior to treatment of their malignancies by this assay method will be done. In addition, these patients will be studied at intervals postoperatively to determine whether these defects preceded the development of cancer and whether a persistent abnormality of macrophage activity is indicative of persistent tumor. The assay is also being incorporated into an immunotherapy regimen. Preliminary observations indicate that BCG stimulates macrophage activity. The response of macrophages to BCG may therefore select those patients likely to benefit from this form of immunotherapy.

Project Description:Part VIII. Cellular Immunity in Cured Cancer PatientsObjectives:

To quantitate cellular immunity in cured cancer patients utilizing in vitro reactivity to phytohemagglutinin (PHA) and in vivo contact sensitivity to dinitrochlorobenzene (DNCB) and to compare the reactivity of these patients with that of preoperative cancer patients having the same histologic types of tumors.

Methods Employed:

Over 100 patients who had been treated for histologically proved malignancies at the Surgery Branch were studied. The patients were divided into five groups according to the histologic type of tumor excised - head and neck squamous cancer, pelvic squamous cancer, sarcomas, melanomas, and adenocarcinomas. All patients were in good general health at the time of the study, and each had undergone a physical examination within three months of the study and were considered clinically cured of tumor at the time. The mean interval since treatment of their primary tumor was eight years and the range five to fourteen years.

Lymphocyte reactivity to PHA and skin contact sensitivity to DNCB were determined by the methods described in the preceding parts of this section. The data derived from these patients were compared with that obtained from study of the preoperative patients and the normal volunteers who were studied during the same time interval. The data derived from these patients have been described in the previous parts of this section.

Major Findings:

The lymphocyte reactivity of patients cured of cancer correlated with tumor histology. Among patients previously treated for squamous carcinomas of the head and neck region and female pelvic organs, there was an increased incidence of patients with low reactivity similar to that found in preoperative patients. Among patients previously treated for sarcomas, melanomas, and adenocarcinomas, the incidence of high reactivity exceeded that of the normal population. Comparison of the high reactivity of cured sarcoma and melanoma patients with the low reactivity of their preoperative counterparts showed a statistical difference of $p < .05$ for the sarcoma patient groups and $p < .01$ for the melanoma patient groups. The effect of the serum of the cancer patients on the reactivity of normal lymphocytes also correlated with tumor histology and the findings were similar in both preoperative and cured patients. In both groups serum from patients with squamous carcinomas and sarcomas significantly inhibited normal lymphocyte reactivity, while serum from patients with melanomas and adenocarcinomas did not have an inhibitory effect.

The distribution of DNCB reactivities among patients cured of cancer also correlated with tumor histology. Among patients with sarcomas, melanomas, and adenocarcinomas, the distribution of reactivities did not differ from that of the normal population. By contrast, patients cured of squamous carcinomas of the head and neck region and female pelvic organs continued to show the abnormal reactivity demonstrated in preoperative patients.

Significance to Biomedical Research and Program of the Institute:

The findings of normal cellular immunity among patients with sarcomas, melanomas, and adenocarcinomas suggest that patients previously treated for tumors of these histologic types who have impaired cellular immunity may be at high risk for having residual tumor. If greater effectiveness of radiotherapy, chemotherapy, and immunotherapy is achieved in patients with relatively small tumor burdens, an important application of the assays used may be for the identification of patients who have residual tumor before it is clinically manifest, to allow earlier institution of therapy. The persistence of immune defects in cured squamous cancer patients provokes questions concerning the role of genetic and environmental factors in the genesis of these tumors. For instance, are these patients from a cohort of the population with pre-existing and perhaps genetically determined abnormalities of thymus-dependent lymphocyte function? Do then the pre-existing immune defects predispose to cancer by increasing the susceptibility to environmental carcinogens? Is a pre-existing immune impairment a prerequisite for induction of cancer by agents which have been linked to squamous carcinogenesis? The high incidence of impaired cellular immunity in squamous cancer patients may also serve as a rational basis for selection of radiotherapy or surgery for lesions which are appropriately treated by either modality. In view of the evidence for an immunosuppressive effect of immunotherapy, patients with impaired immunity might be selected for surgical treatment, while those with normal reactivity may be more favorable candidates for radiotherapy.

Proposed Course:

Serial assays of cellular immunity in patients treated for sarcomas, melanomas, and adenocarcinomas are in progress to determine whether those patients with low reactivity revert to higher reactivity after tumor removal or, conversely, if patients with low reactivity fail to be cured despite definitive surgery. To further elucidate the factor accounting for the persisting immune defects in patients cured of squamous carcinomas, investigation of the genetic profiles of patients with these histologic types of tumors by determination of HLA genotypes is in progress.

Project Description:

Part IX. Correlations of Lymphocyte Reactivity and Tumor Histology with Genetic Profiles in Cancer Patients

Objectives:

To determine the relationship between in vitro lymphocyte reactivity and tumor histology of cancer patients and the HLA types of the patients.

Methods Employed:

The cancer patients studied were those admitted to the protocols described in the previous parts of this section. The normal controls were Blood Bank volunteers who had determination of their HLA type at the time of blood donation. In vitro lymphocyte reactivity to PHA was determined as described in preceding parts of this section. Both preoperative and cured cancer patients were studied. HLA typing was performed by the microcytotoxicity assay.

Major Findings:

After completion of the HLA typing of over 200 cancer patients, analysis of the data revealed that patients with squamous cancer of the head and neck region and female pelvic organs had an increased incidence of HLA-8 ($p < .05$). The HLA-8 prevalence among patients with tumors of other histologic types did not differ from that of the normal controls. Correlations between the presence of HLA-8 and the level of lymphocyte reactivity were then made. Among normal controls, patients who were HLA-8 positive had lower lymphocyte reactivity than those who were HLA-8 negative. The relatively small number of volunteers thus far studied and the small differences obtained revealed a statistical difference between these two groups that is significant at the $p < .09$ level. Study of larger numbers of normal controls in this manner will continue with the anticipation that a greater level of statistical significance will be achieved. Among patients with non-squamous carcinomas, those who were HLA-8 positive had a statistically significant lower incidence of lymphocyte reactivity than those who were HLA-8 negative ($p < .05$). Among patients with squamous carcinomas who have the lowest level of lymphocyte reactivity of any group studied there was no difference in the reactivities of those patients who were HLA-8 positive and those who were HLA-8 negative.

Significance to Biomedical Research and Program of the Institute:

These findings indicate a correlation between the HLA-8 allele and immune reactivity among normal volunteers and patients with non-squamous carcinomas. This is the first evidence for correlation between a genetic factor and immune reactivity. This correlation should serve as a stimulus to further define the relationship between genetic factors and immune reactivity. Such correlations have important implications in the study of the epidemiology and approaches to treatment of cancer patients. It further may provide insight into the mode of action of putative carcinogens in cancer induction. The increased prevalence of HLA-8 positive patients among those with squamous carcinomas suggests that this genetic factor is implicated etiologically in the induction of squamous carcinomas.

Proposed Course:

Studies now in progress with collaborating units investigate the serum viral antibody levels to a panel of viruses that may be related etiologically in the induction of cancer. Correlations will be made with the titers to these antibodies in both preoperative and cured cancer patients and HLA phenotypes. Expansion of the number of patients studied by these immunologic and genetic profiles is in progress to determine the effect of the presence of HLA-8 and to determine whether the presence of HLA-8 affects prognosis after definitive tumor therapy.

Publications:

Catalona, W.J., Taylor, P.T., and Chretien, P.B.: Quantitative dinitrochlorobenzene contact sensitization in a normal population. Clin. Exp. Immunol. 12: 325-333, 1972.

Cullen, B.F., Chretien, P.B., and Leventhal, B.G.: The effect of lignocaine on PHA-stimulated human lymphocyte transformation. Brit. J. Anaesth. 44: 1247-1252, 1972.

Catalona, W.J., Sample, W.F., and Chretien, P.B.: Lymphocyte reactivity in cancer patients: Correlation with tumor histology and stage. Cancer 31:65-71, 1973.

Chretien, P.B., Matthews, W., Jr., and Twomey, P.L.: Serum ribonucleases in cancer: Relation to tumor histology. Cancer 31:175-179, 1973.

Chretien, P.B., Crowder, W.L., Gertner, H.R., Sample, W.F., and Catalona, W.J.: Correlation of preoperative lymphocyte reactivity with the clinical course of cancer patients. Surg. Gynec. Obstet. 136:380-384, 1973.

Catalona, W.J., and Chretien, P.B.: Abnormalities of quantitative dinitrochlorobenzene sensitization in cancer patients: Correlation with tumor stage and histology. Cancer 31:353-356, 1973.

In Press:

Twomey, P.L., and Chretien, P.B.: Cellular immunity in cured cancer patients. Cancer.

Chretien, P.B., and Ketcham, A.S.: Quantitation of immunologic defects in cancer patients. In Seventh National Cancer Conference Proceedings.

Cullen, B.F., and Chretien, P.B.: Ketamine and in vitro lymphocyte transformation. Anesthesia and Analgesia.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3818

Title: Malignancy Associated Cellular and Serum Abnormalities

Principal Investigator: P.B. Chretien, M.D.

Section: Office of the Chief

Lab/Branch: Surgery Branch

Goal: To develop and determine the efficacy of in vivo and in vitro assays that quantitate immune reactivity in cancer patients.

Approach: Techniques for quantitating cellular reactivity have been developed utilizing phytohemagglutinin (PHA) in an in vitro assay of lymphocyte reactivity and dinitrochlorobenzene (DNCB) in an in vivo test of immune competence. Prospective studies of patients receiving definitive surgical treatment of their malignancies have been devised to determine the value of these assays as predictors of response to therapy. Additional in vitro assays that have been applied in these prospective studies include serum inhibitors of lymphocyte reactivity, serum ribonuclease levels, blood macrophage function, and genetic profiles.

Progress: The studies show that both in vitro reactivity to PHA and in vivo reactivity to DNCB quantitate cellular immunity in cancer patients and, furthermore, that the two assays yield comparable results. Both correlate with prognosis after definitive surgery in patients with sarcomas, melanomas, and adenocarcinomas. Patients with squamous carcinomas, however, have persisting immune defects, both cellular and humoral, despite being cured. Investigation of HLA types in cancer patients reveals that patients with squamous carcinomas have an abnormally high frequency of HLA-8. Furthermore, patients with non-squamous cancer and HLA-8 genotypes have lower in vitro lymphocyte reactivity than patients with non-HLA-8 genotypes. Analysis of serum ribonuclease levels and blood macrophage function reveals defects in cancer patients that are not present in cured patients.

Significance for Cancer Research (NCP Objective 5, Approach 4): The findings in these studies indicate that the assays evaluated provide insight into the tumor status of cancer patients. They are, therefore, of potential value as monitors of response to therapy, as a basis for the selection of combinations of therapeutic modalities, as a means of determining dose-rate regulation of cancer therapy that will preserve immune competence, and as detectors of residual disease after treatment before it is clinically manifest. These applications of the assays have the potential for increasing the cure rates of cancer patients with the use of presently available treatment modalities.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-3819
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Study of Cancer Immunology and Immunotherapy in Laboratory Animals

Previous Serial Number: Same

Principal Investigators: G. H. Myers, Jr., M.D., L. Schour, M.D., and N. Javadpour, M.D.

Other Investigators: M. S. Soloway, M.D., J. W. Futrell, M.D., N. L. Albright, M.D., R. P. Faraci, M.D., and W. J. Pendergrast, Jr., M.D.

Cooperating Units: Laboratory of Pathology, National Cancer Institute

Man Years:

Total: 4-1/2
Professional: 2
Others: 2-1/2

Project Description:

- Part I. The Influence of Regional Lymphatics on Tumor Immunity
- Part II. Regional Lymphadenectomy and the Role of Regional Lymphatics in Cancer Immunity
- Part III. Hormonal Therapy of Renal Carcinoma in Mice
- Part IV. Humoral Antibody Response to MCA-Induced Sarcomas
- Part V. Preferential Splenic Metastases in Parabiatic Animals
- Part VI. Effect of Prior BCG Immunization on Melanoma Growth in Mice
- Part VII. The Burn Scar as an Immunologically Privileged Site
- Part VIII. Evaluation of Urinary Cytology as an Indicator for Bladder Necplasia in Mice
- Part IX. Transformation of Hamster Urothelium by SV40 and Its Possible Prevention and Treatment by Fetal Antigen

Project Description:

- Part I. The Influence of Regional Lymphatics on Tumor Immunity

Objectives:

The regional lymphatics are known to play an important role in rejection of skin allografts as demonstrated by experimental work using alymphatic skin pedicles in guinea pigs. Skin allografts on such pedicles will survive indefinitely. Animals with a pedicle graft in place will reject another graft from the same donor in a first-set fashion. Not only is the second graft rejected, but the first graft on the pedicle is also rejected, thereby establishing the presence of intact efferent arc of the immune system. An alymphatic pedicle will be used to study the effect of regional lymphatics on tumor allografts and tumor isografts.

Methods Employed:

Two methylcholanthrene tumors induced in strain 2 guinea pigs will be used as allografts and isografts. These will be injected into strain 2 and strain 13 inbred animals. Alymphatic skin pedicles in each animal will be challenged intradermally with various tumor cell doses. The same model will be used to study immunotherapy and adoptive transfer of tumor immunity in guinea pigs.

Major Findings:

Tumor allografts injected into alymphatic skin pedicles grew to 3 cm., but all eventually regressed. Tumors grew considerably larger in the pedicles than did tumors resulting from allogeneic tumor cell injection into intact skin. Animals previously bearing a tumor allograft demonstrated immunity upon rechallenge. This immunity could be transferred to other animals by the use of spleen cells. In contrast, tumor isografts in alymphatic pedicles grew to 6 cm in size resulting in death of the animal. This occurred with a cell dose which never produced progressively growing tumor in the intact skin. When a pedicle bearing a tumor isograft was excised early in the course of its growth, subsequent rechallenge of the animal with syngeneic tumor demonstrated immunity.

Significance to Biomedical Research and Program of the Institute:

Some investigators have advocated leaving local lymph nodes in place during the course of cancer surgery in order to preserve the patient's immune defense. Findings in this study suggest that regional lymphatics are important for the early initiation of tumor growth, but that once this tumor obtains a certain size, it is no longer under the influence of the local immune system. Once a tumor is large enough to be clinically detected, our findings suggest that the patient has already been sensitized systemically and would not benefit by leaving the local lymph nodes intact, especially if these may contain metastatic tumor.

Proposed Course:

Further Investigations will be carried out using the alymphatic pedicle in guinea pigs to better understand the role of regional lymphatics in tumor growth as well as the effect of surgical manipulation of regional lymphatics in tumor-bearing animals.

Project Description:Part II. Regional Lymphadenectomy and the Role of Regional Lymphatics in Cancer ImmunityObjectives:

Increasing importance has been attached to the role of the regional lymph nodes in the expression of a host's ability to resist tumor growth. Some have suggested that regional lymphadenectomies significantly alter the host's ability to mount an immune response to a tumor challenge. Studies with certain murine tumor systems have suggested that perhaps patients undergoing surgery for cancer should have their local lymph nodes left intact so that their immune response to tumor is not significantly altered. The purpose of this study was to determine what effect regional lymphadenectomy would have upon the host's ability to development concomitant immunity to tumor-specific transplantation antigens.

Methods Employed:

Two hundred and twenty inbred Balb/c mice were randomly inoculated in the right hind limb with 1×10^5 viable isologous fibrosarcoma cells from a methyl-cholanthrene-induced tumor. Twenty mice served as unoperated controls. The remaining 200 mice were divided into four groups of 50 mice each and challenged with 1×10^5 tumor cells in the left hind limb on days 3, 7, 14, and 21 after initial right limb challenge. Prior to the second tumor challenge, 20 mice underwent right popliteal and inguinal lymphadenectomy and 10 mice underwent sham operation. The remaining 20 mice served as controls with 10 additional mice serving as controls for the tumor cell dose used for each challenge.

Major Findings:

Significant concomitant tumor immunity was demonstrated by failure of the second tumor challenge to grow (0-10%) in those mice whose lymphadenectomy was performed on days 7, 14, and 21, as well as in the sham operated and non-lymphadenectomized controls. In contrast, a significant lack of concomitant immunity occurred in those mice whose lymphadenectomy was done on day 3. Forty-two percent of this group grew left and right limb tumors.

Significance to Biomedical Research and Program of the Institute:

This study, along with other studies in guinea pig alymphatic skin pedicles, suggests that although regional lymph nodes are important for early host recognition of tumor antigens, their later removal does not adversely alter the host's ability to mount a tumor-specific immune response.

Proposed Course:

Studies will be continued to further clarify the role which the regional lymph nodes play in the host's response to this tumor.

Project Description:

Part III. Hormonal Therapy of Renal Carcinoma in Mice

Objectives:

To study the effect of hormonal therapy on a spontaneously arising renal adenocarcinoma in Balb/c mice.

Methods Employed:

Balb/c female mice received intramuscular injection of 1×10^4 viable renal adenocarcinoma cells in the right hind limb. Hormonal therapy was begun on the fifth day following tumor inoculation and consisted of either cortisone, progesterone (Provera), testosterone, or diethylstilbestrol.

Major Findings:

Cortisone and progesterone had no effect on growth of the murine adenocarcinoma. Testosterone and diethylstilbestrol both inhibited the renal tumor transplant. Inhibition was greater with diethylstilbestrol than with testosterone.

Significance to Biomedical Research and Program of the Institute:

Treatment for metastatic renal cell carcinoma has usually been disappointing. Hormonal therapy has been proposed in such patients based on evidence derived from other studies employing a renal tumor induced in the hamster by prolonged administration of diethylstilbestrol. It is felt that the tumor-host system used in this study was superior to the hamster model because the tumor was a spontaneous tumor which resembled human renal cell carcinoma, grossly and histologically, and which arose in an inbred strain of mice allowing transplantation without fear of tumor inhibition on the basis of histocompatibility antigens.

Proposed Course:

This experimental tumor will be used to further evaluate hormonal therapy of renal cell carcinoma and also be used to evaluate various chemotherapeutic agents.

Project Description:

Part IV. Humoral Antibody Response to MCA-induced Sarcomas

Objectives:

To detect tumor antigens of a methylcholanthrene-induced sarcoma by complement fixation.

Methods Employed:

Fifteen adult female strain 2 guinea pigs were immunized by weekly intradermal injections of 1×10^6 viable MCA-A tumor cells. This is a methylcholanthrene-induced liposarcoma which arose subcutaneously in a strain 2 guinea pig. The tumor has been shown to possess tumor-specific transplantation antigens. Although an intramuscular injection of 1×10^5 viable MCA-A tumor cells always cause a lethal tumor, an inoculation of over 1×10^7 viable tumor cells was required to produce progressive intradermal tumor. Serum was obtained before, during, and after immunization. Tumor antigens were prepared from freshly excised tumor or from tissue culture for use in a modified micro-complement fixation test.

Major Findings:

Antitumor antibody was detected after the second injection. After the third immunization, the mean antibody titer rose to 1/64 and remained elevated with successive weekly injections of viable tumor cells. Sera from normal strain 2 guinea pigs did not combine and fix complement in the presence of MCA-A tumor antigens. Animals that had repeated intradermal immunizations were later challenged intramuscularly with 1×10^4 tumor cells and all were completely protected. In contrast, unimmunized animals given the same inoculum developed large lethal tumors. Sera from strain 2 guinea pigs given repeated intradermal inoculations of viable MCA-A tumor cells contained a complement fixing antibody that reacted specifically with antigens prepared from the immunizing tumor but not with antigens from normal tissue.

Significance to Biomedical Research and Program of the Institute:

Previous attempts to detect tumor antigens capable of eliciting a humoral response of a chemically-induced tumor have been limited primarily to the murine systems, and have usually failed. Repeated intradermal injection of a subgrowth tumor inoculum has been shown to be effective as a method of immunotherapy or immunoprophylaxis against chemically induced tumors of syngeneic guinea pigs. The success of such intradermal therapy has not been thought to be related to a humoral immune response. However, the results of this study demonstrate that the guinea pig can mount a humoral immune response to tumor-specific antigens after intradermal inoculation of viable isologous tumor cells.

Proposed Course:

Further studies are underway to determine the relationship of the complement fixing antigen to the tumor-specific transplantation antigens known to induce transplantation immunity.

Project Description:Part V. Preferential Splenic Metastases in Parabiotic Animals

Objectives:

Experiments have been carried out with a spontaneously arising reticulum cell sarcoma which spreads preferentially to spleen. Studies were carried out to see if this tumor would metastasize across a parabiotic union and to determine if the splenotrophism of this tumor would be manifest in parabiotic animals.

Methods Employed:

Normal C3H female mice or splenectomized mice were placed in parabiosis with normal C3H mice. Fourteen days later, the splenectomized animal or a normal animal of a normal-parabiotic pair received 10^6 reticulum cell sarcoma cells intramuscularly. Twenty-one days later, animals were sacrificed and examined for presence of gross metastatic tumor. In a second experiment, the single animals (control or splenectomized) received 10^6 cells intramuscularly. Other animals received 10^6 cells intramuscularly and were then placed in parabiosis either with normal animals or with splenectomized animals. Twenty-three days later, animals in all groups were sacrificed and examined for the presence of metastatic tumor.

Major Findings:

Injection of tumor cells into animals in parabiosis for 14 days was followed by splenic metastases in 100% of the animals. There were no extra splenic metastases. Injection of tumor cells into the splenectomized member of a pair of animals placed in parabiosis 14 days earlier, produced splenic metastases in 100% of animals with no extra splenic metastases. Normal single animals receiving tumor cells showed splenic metastases in 100% with metastases to the liver in 36% and to the kidney in 9%. The incidence of metastases in splenectomized animals was 93% to liver and 61% to kidney. When normal animals were injected with tumor cells and then placed in parabiosis with another normal animal, splenic metastases were found in all animals with metastasis to the liver in 2 of 12 injected animals and metastasis to the kidney in 1 of 12 injected animals. When a single animal was injected with tumor cells and then placed in parabiosis with a splenectomized animal, tumor was found in the spleens of 100% of the injected animals and 100% of the uninjected member of the pairs. No extra splenic metastases were seen in the injected member of this pair, but liver metastases occurred in 22% of the uninjected animals and 11% kidney metastases were seen in this group.

Significance to Biomedical Research and Program of the Institute:

These studies demonstrate that metastases of a reticulum cell sarcoma induced by injection of tumor cells intramuscularly in one animal, can spread across a parabiotic union to involve a second animal. The marked splenotrophism of this tumor results in selective metastasis to the spleen of a normal animal placed in parabiosis with a tumor-bearing animal. The splenotrophism of this tumor, as well as its ability to be transmitted across a parabiotic union, should provide an excellent model for the study of immunologic factors influencing preferential metastases.

Proposed Course:

Various methods of altering the immunoresponsiveness of normal animals placed in parabiosis with animals bearing this splenotrophic tumor will be studied. Immunosuppression by x-irradiation and cortisone administration will be carried out in one or both members of the parabiotic pairs.

Project Description:Part VI. Effect of Prior BCG Immunization on Melanoma Growth in MiceObjectives:

Intralesional injection of BCG into melanoma nodules in human patients had been reported to cause regression of tumor. The purpose of this study was to determine if prior immunization with BCG would inhibit tumor growth following inoculation of mice with melanoma cells.

Methods Employed:

Balb/c mice were immunized with four weekly intramuscular injections of 0.1 cc of BCG followed by an intraperitoneal injection of 0.1 cc of BCG. Skin testing was accomplished by foot pad and intradermal injection of PPD one week after the last BCG injection. Mice with positive skin tests were used for the remainder of the experiment. Control and BCG immune mice were then divided into three groups, respectively, and received 10^4 to 10^6 Cloudman melanoma cells intramuscularly. Tumor incidence seven weeks following tumor challenge was measured.

Major Findings:

Overall tumor incidence in the BCG immune group was 34% lower than in the control group ($p < .01$). Animals receiving the highest tumor doses (10^6) showed the greatest difference in tumor incidence between the BCG immune and control groups (45%).

Significance to Biomedical Research and Program of the Institute:

The decreased tumor incidence of the BCG immune animals may result from non-specific immune stimulation due to BCG immunization, or from specific rejection of melanoma cells due to sharing of common antigens by tubercle bacilli and melanoma. An understanding of the mechanism would have implications for the use of BCG in human tumors.

Proposed Course:

In vitro studies are underway to investigate the mechanism of the decreased tumor incidence noted in BCG immunized animals.

Project Description:Part VII. The Burn Scar as an Immunologically Privileged SiteObjectives:

To test the hypothesis that a burn scar may act as a privileged site where host recognition of tumor-specific antigens is delayed, allowing the development and progressive growth of antigenically foreign tumor cells, thereby escaping the normal immunal surveillance mechanism.

Methods Employed:

Standardized full-thickness burns were created on the flanks of inbred strain 2 female guinea pigs, requiring an average of 85 for re-epithelialization. An alymphatic skin pedicle was created on the flanks of other animals. A methyl-cholanthrene-induced liposarcoma previously shown to contain tumor-specific transplantation antigens was used in all experiments. Data from previous experiments indicates that intradermal injection of greater than 1×10^7 viable tumor cells is required to produce a progressively growing tumor. 5×10^5 tumor cells were injected intradermally into four control animals with intact skin, the healed burn scars of 11 animals, and intradermally into the surgically created alymphatic skin pedicles of six animals. Random animals in each group underwent excision of the inoculation site on day 15 following injection. These and control animals were re-challenged intramuscularly with 1×10^3 tumor cells.

Major Findings:

5×10^5 tumor cells inoculated intradermally into normal guinea pigs with intact lymphatics were consistently rejected without the development of palpable tumor. When an identical inoculum was injected intradermally into alymphatic skin pedicles, the tumors grew progressively resulting in death of the animals. Injection of an identical inoculum into burn scars of 11 animals was followed by growth of large tumors in seven (64%), which if not excised, proved lethal. Animals with progressively growing "alymphatic pedicle tumors" and burn scar tumors eventually were sensitized to tumor-specific transplantation antigens as evidence by protection against an IM challenge with the same isologous tumor following excision of the pedicle tumors or burn scar tumors.

Significance to Biomedical Research and Program of the Institute:

The fact that burn scar and the alymphatic skin pedicle both allow normally rejected tumor cells to grow indicates that both of these areas may act as an immunologically privileged site where recognition of tumor antigens is delayed sufficiently for a tumor to grow to a critical size, at which time immune mechanisms are no longer effective in preventing continued tumor progression. These findings are consistent with other information that indicates the immunological defense mechanisms are operative in the normal individual to protect against the disease and to allow for the recognition rejection of "non-self." This

immuno-surveillance mechanism may be of prime importance in the destruction of foci of cells transformed by spontaneous mutation or by viral or chemical carcinogens. Growth of syngeneic tumor cells in an area surgically deprived of afferent lymphatic drainage suggest that any process resulting in alterations of local afferent lymphatic vessel integrity may potentially enhance the likelihood of tumor development in such an area.

Proposed Course:

Further study of surgical scars is planned to determine if absence of afferent lymphatic channels is the mechanism responsible for the reported localization of circulating tumor cells in surgical scars.

Project Description:

Part VIII. Evaluation of Urinary Cytology as an Indicator for Bladder Neoplasia in Mice

Objectives:

To determine the value of urinary cytology findings in mice with induced bladder tumor.

Methods Employed:

C3H and C57B1 mice were randomly divided into control and experimental groups. Experimental groups received a diet containing 0.1% FANFT by weight mixed with regular food and the control groups received regular diet alone. At monthly intervals, five mice were sacrificed from each group and bladders were removed and studied histologically. Urine samples from each of the four groups were obtained monthly. Urine was filtered through a millipore filter and filters were stained with the Papinicolaou technique.

Major Findings:

The efficacy of a diet containing FANFT in induction of bladder neoplasms in mice was confirmed. Nine to 10 months following initiation of the carcinogen, 81% of the C3H mice had bladder tumors. It required 11 months of carcinogen feeding for 70% of the C57B1 mice to develop bladder neoplasms. Atypical cells were observed in the urine from the C3H mice beginning five to seven months after initiation of the carcinogen. Clearly neoplastic cells were rarely identified. The first urine sample from the mice showed 30 red blood cells per cubic millimeter. This persisted throughout almost all subsequent samples. Microhematuria was first observed in the urine from the C57B1 mice at seven months and persisted through subsequent specimens.

Significance to Biomedical Research and Program of the Institute:

The presence of red blood cells in the urine was an accurate indicator of the presence of bladder neoplasms in mice with this experimental tumor. In this

tumor system, one cannot rely solely on the identification of neoplastic cells in screening urine for bladder cancer.

Proposed Course:

Further use of this carcinogen is planned to induce bladder tumors in mice and initiate experimental therapeutic regimes, including radiation, chemotherapy, and immunotherapy. The effectiveness of such treatments will be monitored by urinary cytology findings as well as by other parameters.

Project Description:

Part IX. Transformation of Hamster Urothelium by SV40 and Its Possible Prevention and Treatment by Fetal Antigen

Objectives:

Since the urothelial cancers are usually multicentric and recurrent, a possible viral etiology has been suggested. Due to the lack of technique to isolate the urothelium from the underlying lamina propria, it has not been possible to culture and transform the urothelium.

Methods Employed:

Inbred hamsters have been used for cystectomies. A new simple technique has been devised to separate the mucosa (urothelium) of the bladder by injection of 1-2 cc of normal saline in the lamina propria and subsequently dissect the urothelium for tissue culture. After the cell lines have been established, the urothelium have been transformed in vitro by addition of SV40.

It has been planned to transplant the transformed urothelial tissue in syngeneic hamsters to establish the tumor. The fetal antigen prepared from the 10-12 day old embryo has been used to immunize a group of syngeneic hamsters. Attempts will be made to transplant the already established tumor into the "immunized hamsters." A group of hamsters with injection of the plain tissue as well as other groups of hamsters will serve as appropriate controls.

Major Findings:

Thus far, 40% of the urothelium has been transformed. The cross-reactivity between the T-antigen and fetal antigen will be detected.

Significance to Biomedical Research and Program of the Institute:

The lack of technique to isolate the urothelium has been a major problem in culturing and transforming the urothelium by oncogenic viruses. As a result, the cross-reactivity and antigenicity of these cancers have not been studied.

Proposed Course:

We will continue to establish the urothelial tissue of urinary tract in experimental animals. Also continue the study of cross reactivity between T-antigens of the oncogenic viruses and fetal antigens in order to detect the antigenicity of the tumor and feasibility of immunizing the laboratory animals against these types of carcinomas.

Publications:

Futrell, J.W. and Myers, G.H., Jr.: Role of regional lymphatics in tumor allograft rejection. Transplantation 13:551-557, 1972.

Albright, N.L. and Myers, G.H., Jr.: Specific antigens of a chemically induced tumor: Detection by complement fixation. J. Natl. Cancer Inst. 49:295-297, 1972.

McCoy, J.L., Ting, R.C., Morton, D.L., and Law, L.W.: Immunological and virological studies of a non-producer tumor induced by murine sarcoma virus (Harvey). J. Natl. Cancer Inst. 48:383-391, 1972.

Soloway, M.S., Pendergrast, W.J. and Myers, G.H., Jr.: Effect of regional lymphadenectomy on development of concomitant immunity. Surg. Forum 23:101-102, 1972.

Futrell, J.W. and Myers, G.H., Jr.: The burn scar as an "immunologically privileged site." Surg. Forum 23:129-131, 1972.

Futrell, J.W. and Myers, G.H., Jr.: Regional lymphatics and cancer immunity. Ann. Surg. 177:1-7, 1973.

Soloway, M.S., Myers, G.H., Jr., Marrone, J.C., Del Vecchio, P.R., and Malmgren, R.A.: Evaluation of urinary cytology as an indicator for bladder neoplasia in mice. J. Urol. 109:249-252, 1973.

Soloway, M.S. and Myers, G.H., Jr.: The effect of hormonal therapy on a transplantable renal cortical adenocarcinoma in syngeneic mice. J. Urol. 109:356-361, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3819

Title: Study of Cancer Immunology and Immunotherapy in Animals

Principal Investigators: G.H. Myers, Jr., M.D., and L. Schour, M.D.

Section: Office of the Chief

Lab/Branch: Surgery Branch

Goal: To evaluate immunologic factors relating to the development of successful immunotherapy for experimental and human tumors.

Approach: The immunological aspects of genitourinary tumors are being studied in inbred mice. Bladder tumors induced in two strains of inbred mice are being used to study the effectiveness of hormones in the treatment of these tumors. Exfoliative urinary cytology has been unable to detect bladder cancer in mice. However, microscopic hematuria correlated well with the development of early bladder cancer. Preparation of alymphatic pedicles in guinea pigs followed by implantation of MCA-induced sarcomas, has suggested that regional lymphatics are important for the early initiation of tumor growth, but that once a tumor attains a certain size, it is no longer under the influence of the local immune system. Balb/c mice are being used to study the effect of regional lymphadenectomy upon the host's ability to develop concomitant immunity to tumor-specific transplantation antigens.

Progress: Findings to date suggest that, although regional lymph nodes are important for early host recognition of tumor antigen, their later removal does not adversely alter the host's ability to mount a tumor-specific immune response. Repeated intradermal injection of a sub-growth tumor inoculum has been shown to be effective as a method of immunotherapy or immunoprophylaxis against chemically-induced tumors of syngeneic guinea pigs. Complement fixing antibody was detected in these animals. Utilizing a splenotrophic tumor arising in C3H mice, studies have demonstrated that this tumor can metastasize across a parabiotic union. The marked splenotrophism of this tumor is demonstrated by preferential metastases of this tumor to be spleen of the uninoculated member of the parabiotic pair. Prior BCG immunization has been found to reduce the incidence of tumor development in Balb/c mice inoculated with melanoma cells. Studies of kidney, prostate, and testis tumors are being carried out using human cell lines grown in tissue culture.

Significance to Cancer Research (NCP Objective 4, Approach 2): These studies have a direct relationship to the development of methods for detection of human cancers and the development of immunotherapy for such tumors.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-3820
1. Surgery Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cellular Immunity to Tumor Specific Transplantation Antigens

Previous Serial Number: Same

Principal Investigators: S.A. Wells, Jr., M.D., M.H. Cohen, M.D., Ph.D.,
and A.M. Cohen, M.D.

Other Investigators: Y.H. Pilch, M.D., B.R. MacPherson, M.D., R.C. Millar,
M.D., and A.S. Ketcham, M.D.

Cooperating Units: None

Man Years:

Total: 3-1/2
Professional: 1-1/2
Other: 2

Project Description:

Part I. Cellular Immunity and "Blocking Factors" to Tumor Specific Transplantation Antigens in Animal Systems

Part II. Cellular Immunity and "Blocking Factors" in Patients with Skeletal and Soft Tissue Sarcomas as Studied In Vitro with Cell Mediated Cytotoxicity Assays

Part III. Immune Cytolysis In Vitro and its Correlation with Immunity

Project Description:

Part I. Cellular Immunity and "Blocking Factors" to Tumor Specific Transplantation Antigens in Animal Systems

Objectives:

To study in vitro the lymphocyte response to growing tumors in strain 2 guinea pigs. To study the effect on cell mediated immunity of humoral "blocking factors."

Methods Employed:

Host immunity to a growing antigenic solid tumor was studied with a transplanted

methylcholanthrene-induced sarcoma (MCA-25) in inbred female strain 2 guinea pigs. Tumor specific cellular immunity was evaluated in vivo by delayed cutaneous hypersensitivity reactions to a transplantation antigen extract of the tumor and evaluated in vitro by a splenic lymphocyte-mediated cytotoxicity. The assay for in vitro lymphocyte-mediated cytotoxicity was performed by labelling the tissue culture target cells with I^{125} -iododeoxyuridine (IDU), a DNA label. After the addition of immune lymphocytes to the tissue culture, the loss of radioactivity from the monolayers culture was used as an index of lymphocyte-mediated cytotoxicity.

Major Findings:

Animals receiving viable MCA-25 cells intramuscularly demonstrated tumor specific cellular cytotoxicity. They developed progressing tumors. The degree of tumor specific cellular cytotoxicity was similar to that of other guinea pigs who had been immunized to sarcoma cells by temporary tumor growth followed by amputation. The most striking finding was that serum from animals with palpable tumors completely inhibited the sarcoma specific cell mediated cytotoxicity reaction. The same serum did not inhibit cellular cytotoxicity directed against a different methylcholanthrene-induced tumor. Thus, the addition of serum from animals with progressively growing tumors abrogated a lymphocyte-mediated cytotoxicity, indicating the presence of a "blocking factor" in the serum from animals with progressing tumors.

Significance to Biomedical Research and Program of the Institute:

The findings of tumor specific cellular cytotoxicity during the growth of transplanted methylcholanthrene-induced sarcoma in guinea pigs agrees with other in vitro studies of concomitant tumor specific cell mediated immunity in the presence of growing tumors induced by either Moloney sarcoma virus, spontaneous mouse mammary tumors, polyoma virus-induced rat tumors, other methylcholanthrene-induced tumors, and tumors induced by plastic discs. In some systems, tumor specific cellular cytotoxicity has not been present in animals bearing large tumors. It is possible in these cases that the supply of splenic effector cells has been "exhausted" in the presence of a large growing tumor. The use of I^{125} IDU as a label is advantageous in that there is minimal leak of the label from live cells. After immune cytolysis, the label is not re-utilized by adjacent cells. By using a gamma emitter, this label is easier to count than methods which use other isotopes.

The finding of "blocking factors" is in agreement with previous observations about the presence of such factors in animals and patients with progressive tumors. This assay is well adapted for study of this phenomenon.

Proposed Course:

This cell mediated immune system will be used to measure cell mediated immunity in human tumor systems. We plan to assay serially patients who will be receiving immunotherapy against cancer. We also hope to measure changes in levels of "blocking factors" during the course of immunotherapy.

Project Description:

Part II. Cellular Immunity and "Blocking Factors" in Patients with Skeletal and Soft Tissue Sarcomas as Studied In Vitro with Cell Mediated Cytotoxicity

Objectives:

The main purpose was to evaluate the presence and degree of cell mediated immunity to human sarcomas, and to assess the presence or absence of "enhancing" or "blocking" factors in the serum of patients in various stages of disease.

Methods Employed:

Patients were investigated who were being evaluated by the Surgery Branch or who had undergone surgery here previously. Lymphocytes from these patients were tested against a human osteogenic sarcoma cell line and against a fibroblast line from the same patient. A human lung epidermoid carcinoma line and a fibroblast culture from the same patient were also used as target cells. The I¹²⁵ IDU labelled target cells were then reacted with patients' lymphocytes and the resulting cytotoxicity was assayed. In addition, serum from patients in various stages of their disease were assayed for their ability to inhibit cell mediated cytotoxicity.

Major Findings:

Sarcoma-specific cellular cytotoxicity was observed in 14 of 18 patients with various skeletal and soft tissue sarcomas. Lymphocytes from these patients were cytotoxic to the osteosarcoma cell line but not to fibroblasts from the same patient. Lymphocytes from patients with melanoma and from recipients of multiple blood transfusions did not react in a control assay. In addition to measuring the cytotoxicity against the sarcoma cell line, the serum from 14 of 16 sarcoma-bearing patients was found to inhibit sarcoma-specific cellular cytotoxicity. This compared to inhibition by one of eight melanoma or carcinoma-bearing patients. Two of 11 sera that inhibited sarcoma-specific cellular cytotoxicity also inhibited lung carcinoma-specific cellular cytotoxicity. Serum from four out of 14 sarcoma patients following radical surgery inhibited the sarcoma-specific cellular cytotoxicity. The presence of inhibitory sera in these sarcoma patients was usually associated with recurrent or metastatic disease.

Significance to Biomedical Research and Program of the Institute:

Skeletal and soft tissue sarcomas carry tumor cell surface antigens which seem to be common between several skeletal and soft tissue sarcomas from different patients. The data referred to above suggests that sarcoma-specific transplantation resistance can be observed against a common human sarcoma cell-surface antigen. As was true in animal systems, sarcoma-specific cellular cytotoxicity was present not only in patients who were free of disease,

but also in patients who were bearing sarcomas. Again, the degree of immunity was similar in the sarcoma-free and the sarcoma-bearing group. The striking difference between the sarcoma-free and sarcoma-bearing patients was the inhibitory effect of serum from tumor-bearing patients. As in the guinea pig system, there appears to be some "blocking factor" which prevents in vitro immune lysis of tumor cells by lymphocytes. The fact that patients with progressive growing tumors have such a factor in their serum is in agreement with the observation by the Hellstroms, Dr. Sjorgren, and others who observe "enhancing" or "blocking" factors in patients' serums. An understanding of these factors may help to provide effective therapy to these patients.

Proposed Course:

Attempts should be made to correlate more fully the presence of cell mediated immunity and "blocking factors" with the patient's clinical course. Attempts to enhance the reactivity of these lymphocytes or to diminish the activity of such "blocking factors" should have a beneficial therapeutic effect. In addition, it should be possible to monitor changes in these modalities with immunotherapy, surgery, or other therapy.

Project Description:

Part III. Immune Cytolysis In Vitro and its Correlation with Immunity

Objectives:

To develop quantitative assay for the measurement of cell mediated immunity using tritiated thymidine to label target cell DNA, followed by addition of lymphocytes and measurement of radioactivity remaining in the tissue culture plates. By utilizing a different technique for assay of cell mediated immunity, it is anticipated that conclusions may be drawn regarding similarities or differences between various techniques measuring in vitro immunity. The interpretation of these differences may be important in distinguishing the relevant from the irrelevant aspects of the in vitro tests, as they apply to the in vivo situation.

Methods Employed:

The DNA of target cells was labelled with tritiated thymidine. The cell plates were then reacted with lymphocytes from immune and normal guinea pigs. Supernatants from cultures containing immune lymphocytes and target cells were tested on other target cell cultures to look for cytolytic factors in the milieu of the attacking lymphocytes. Antibodies were also tested for their ability to produce lysis in this system.

Major Findings:

Both syngeneic and allogenic immune guinea pig lymphocytes produced immune cytotoxicity of target cells of guinea pig sarcomas. Immune serum was not effective in producing cytotoxicity. Immune lymphocytes and target cells fail

to cause lysis of immunologically unrelated target cells in the same culture. Supernatants from cultures containing immune lymphocytes and target cells caused no cytolysis. However, slight cytolysis was demonstrable with "immune" supernatants were mixed with normal lymphocytes and the mixture was reacted with target cells.

Significance to Biomedical Research and Program of the Institute:

The best explanation for the mechanism of cytolysis under these experimental conditions appears to be that target cells are destroyed by direct interaction with immune lymphocytes. Immune serum in this system was not effective in producing cytolysis and immune supernatants were only minimally effective in producing cytolysis after being mixed with normal lymphocytes and then with target cells. These results confirm the ability of the attacking lymphocyte to kill target cells in the guinea pig sarcoma system. It will be necessary to extend these studies to human systems and to correlate the clinical features in order to determine the clinical relevance of these findings.

Proposed Course:

It will be necessary to use a multiphasic screening approach with this assay and other assays mentioned in these reports, in order to determine which will be most closely correlated with clinically significant phenomena, and which are misleading or not clinically relevant. In this case, the inability of immune serum to effect target cells should be evaluated in light of the fact that assays using complement fixation techniques, isotopic antiglobulin techniques, and I¹²⁵ labelled target cells all indicate that immune serum does have an effect against target cells in the animal sarcoma systems.

Publications:

MacPherson, B.R., and Pilch, Y.H.: Immune cytolysis in vitro - A correlate of cellular immunity. J. Surg. Res. 12: 193-198, 1972.

MacPherson, B.R., and Pilch, Y.H.: Cellular cytolysis in vitro: Mechanisms underlying a quantitative assay for cellular immunity. J. Natl. Cancer Inst. 48: 1619-1627, 1972.

Morton, D.L., and Wells, S.A.: Immunobiology of neoplastic disease: Melanoma. In Christopher's Text Book of Surgery. Philadelphia, W.B. Saunders Co., 1972, pp. 542-567.

Cohen, A.M., Ketcham, A.S., and Morton, D.L.: Cellular immunity to a common human sarcoma antigen and its specific inhibition by sera from patients with growing sarcomas. Surgery 72: 560-567, 1972.

Cohen, A.M., Millar, R.C., and Ketcham, A.S.: Host immunity to a growing transplanted methylcholanthrene-induced guinea pig sarcoma. Cancer Res. 32: 2421-2426, 1972.

Cohen, A.M.: Host immunity to growing sarcomas: Tumor-specific serum inhibition of tumor-specific cellular immunity. Cancer 31: 81-89, 1973.

Cohen, A.M., Ketcham, A.S., and Morton, D.L.: Tumor-specific cellular cytotoxicity to human sarcomas: Evidence for a cell-mediated host immune response to a common sarcoma cell-surface antigen. J. Natl. Cancer Inst. 50: 585-590, 1973.

In Press:

Cohen, A.M., Ketcham, A.S., and Morton, D.L.: Specific inhibition of sarcoma-specific cellular immunity by sera from patients with growing sarcomas. Int. J. Cancer

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3820

Title: Cellular Immunity to Tumor Specific Transplantation Antigens

Principal Investigators: S.A. Wells, Jr., M.D., M.H. Cohen, M.D., Ph.D., and
A.M. Cohen, M.D.

Section: Tumor Immunology Section

Lab/Branch: Surgery Branch

Goal: To evaluate in vitro cell mediated immunity to human and animal sarcomas, and to measure "enhancing" or "blocking" factors in the serum of animals and patients in various stages of disease.

Approach: I¹²⁵-iododeoxyuridine (IDU) DNA-labelled tissue culture target cells were studied in guinea pig and human systems. This assay for in vitro lymphocyte-mediated cytotoxicity was used to estimate the degree of immunity of lymphocytes from immunized animals and from animals with progressively growing tumors. Similar studies were carried out in patients with a variety of skeletal and soft tissue sarcomas. Lymphocytes from these patients were tested against autochthonous and allogeneic sarcoma cell lines. In both the guinea pig and the human system, the serum from tumor-bearing cases were tested for their ability to block in vitro cell mediated immunity. The in vitro findings were then correlated with the course of the disease.

Progress: Cell mediated immunity was demonstrated in vitro in both animals and patients with progressively growing sarcomas. However, sera from animals or patients with progressively growing tumors inhibited the cell mediated cytotoxicity reaction. Patients who had undergone a resection of tumor and had subsequent appearance in the serum of blocking factors were usually harboring latent or overt metastatic disease. Studies will be carried out to further correlate the in vitro findings with changes in clinical course.

Significance to Cancer Research (NCP Objective 5B, Approach 6): These studies will help achieve an accurate assessment of the presence, extent, and possible course of existing or resected human cancers. These in vitro measurements may also help delineate "blocking" or "enhancing" substances that favor tumor growth. The changes in in vitro immunity and in the levels of these blocking factors can be correlated with clinical course during therapy of the patient, especially immunotherapy.

Level of Effort: Professional Man-Years: 1-1/2

Serial No. NCI-3808
1. Surgery Branch
2. Tumor Immunology
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Study of Host Factors Associated with Implantation and Growth
of Cancer

Previous Serial Number: Same

Principal Investigators: S.A. Wells, Jr., M.D., and M.H. Cohen, M.D., Ph.D.

Other Investigators: F.C. Melewicz, M.D., J.F. Burdick, J.F., A.M. Cohen,
M.D., B.P. LeMevel, M.D., and J.P. Grant, M.D.

Cooperating Units: None

Man Years:

Total:	3
Professional:	2
Other:	1

Project Description:

Part I. Development of In Vitro Assays of Humoral Immunity

Part II. In Vivo Delayed Hypersensitivity Tumor Specific Reactions
in Animals and Patients

Part III. Immunotherapy of Solid Tumors

Project Description:Part I. Development of In Vitro Assays of Humoral ImmunityObjectives:

Tumor specific antigens have been demonstrated in several viral and carcinogen-induced animal neoplasms. The relative contribution of cell mediated and humoral immunity against these tumors has not been elucidated. In order to facilitate studies in this field, it will be necessary to develop multiple sensitive assays of immunity against transformed cells.

Methods Employed:

Three different techniques were used to assay humoral immunity against tumor cells. The isotopic antiglobulin test has been used in the past for characterizing cell surface antigens of murine SV-40 and polyoma tumors. This test has previously been performed with cells in suspension, but the multiple centrifugations and resuspensions which this technique requires have tended to limit the usefulness of this test. An isotopic antiglobulin microassay was developed using tissue culture monolayers with a simplified washing procedure. The test is analogous to the indirect immunofluorescence test in which antibody is reacted with cells and subsequent antiglobulin is added which attaches specifically to the added antibody. In the indirect immunofluorescence test, this last antiglobulin is labelled with fluorocene, but in the isotopic antiglobulin assay, the antiglobulin is labelled with I^{125} and the enumeration procedures are thereby simplified by using this gamma-emitter.

A second assay which was modified for studies in this area was the I^{125} labelled iodoeoxyuridine (IDU) test. This reagent is incorporated into the DNA of target cells and is a useful tag for these cells since the nonspecific release of the label is very low and since the reutilization after cell death is also very low.

The third assay which was modified for use in assaying humoral immunity was the complement fixation assay which depends in this case on the interaction between antibody and tumor specific antigen followed by the binding of complement to the antigen-antibody complex.

Major Findings:

Using the isotopic antiglobulin assay, it was found that antibody against the tumor specific surface antigens of carcinogen-induced murine sarcomas were detected with high sensitivity. Antibody against the tumor was detected in the serum of animals which have actively growing tumors. Half of the methylcholanthrene-induced tumors that were studied had a common cell surface antigen as detected by this assay.

Using the I^{125} IDU tumor cell label, the microassay for the detection of cyto-

toxic tumor specific antibody revealed that small amounts of serum and cells could be utilized to demonstrate serum mediated immunity against methylcholanthrene-induced sarcomas of mice. The assay was simple, sensitive, and apparently objective. The immune serums were highly cytotoxic at high dilutions against the immunizing tumor cells. At low dilutions the immune serum was only minimally cytotoxic against unrelated methylcholanthrene-induced tumors of mice. This reactivity could represent cross reaction. However, the cytotoxicity could be absorbed from the immune serum only by the tumor cells used for immunization and not by unrelated tumor cells or by normal cells.

Using the micro-complement fixation technique, tumor antigens could be detected against a methylcholanthrene-induced sarcoma of guinea pigs. In these animals, the immunizing cells had been given intradermally. The sera contained a complement fixing antibody that reacted specifically with antigens prepared from the immunizing tumor, but which did not react with antigens from an unrelated methylcholanthrene-induced sarcoma. Similarly, there was no reaction with antigens prepared from normal tissues. A significant complement fixation titer could be detected with antigens prepared from either freshly excised tumor or with antigens prepared from a tissue culture line of the immunizing tumor.

Significance to Biomedical Research and Program of the Institute:

These studies demonstrate a tumor specific antigen in a variety of methylcholanthrene-induced tumors of mice and guinea pigs. Immune serum from these animals is capable of interacting with these tumor cells using this variety of techniques. Cross reaction seems to be more apparent between methylcholanthrene-induced sarcomas of mice than between the methylcholanthrene-induced sarcomas in guinea pigs. These studies emphasize the importance of serum mediated immunity in these systems and emphasize the necessity for monitoring the humoral aspect of immunity in immunotherapy protocols in animals and in humans.

Proposed Course:

These assays will have to be applied to human tumor systems as well as to more animal tumor systems in order to assess the generality of the principles observed. They will constitute useful monitors in immunotherapy protocols. It will be of interest to correlate the clinical course with the changes in the in vitro parameters.

Project Description:

Part II. In Vivo Delayed Hypersensitivity Tumor Specific Reactions
in Animals and Patients

Objectives:

The delayed hypersensitivity reaction as a measure of cell mediated immunity

allows in vivo assessment of tumor immunity in both animals and in man. One method of demonstrating tumor immunity in vivo has been the elicitation of delayed cutaneous hypersensitivity reactions to intradermal injection of tumor cell extracts. The purpose of these studies was to utilize this technique to assess the nature of immunity in guinea pigs against methylcholanthrene-induced sarcomas, to determine the amount of fetal antigen cross reactivity with this system, and to assess in humans the reactivity of patients with carcinoma of the lung and carcinoma of the uterine cervix against appropriate tumor cell extracts.

Methods Employed:

Inbred strain 2 guinea pigs were immunized with irradiated cells of two different methylcholanthrene-induced syngeneic guinea pig sarcomas. They were also immunized against second trimester fetal tissue and then skin tested with tumor membrane extracts and extracts of fetal tissue. These results were correlated with experiments in which the guinea pigs were also challenged with viable tumor cells.

In the human studies, 75 patients with lung cancer and 14 patients with carcinoma of the uterine cervix were tested against standard skin test antigens, as well as DNCB and tumor extracts. As controls, patients with benign lung disease were evaluated. Patients with unrelated tumors were also tested.

Major Findings:

In the guinea pig studies, a cross reaction was observed between fetal antigens in the second trimester fetuses and transplantation antigens of the methylcholanthrene-induced guinea pig sarcomas. Animals immunized against fetal tissue had an increased resistance to tumor cell challenge. The delayed cutaneous hypersensitivity reaction was positive in almost all animals who were immunized with either tumor or fetal tissue and then skin tested against that same antigen. Animals immunized with fetal tissue also had positive skin tests against tumor antigens.

Among patients with lung cancer or cancer of the uterine cervix, about half reacted against either autologous tumor antigen or allogeneic antigen prepared from another tumor of the same histological type. There was a significant incidence of cross reactivity between patients who had cervical cancer, for example, who were being tested with antigen prepared from cervical carcinoma of another patient. The high incidence of cross reactivity suggests the presence of a common antigen in the tumor cells of patients with lung cancer or cervical carcinoma.

Significance to Biomedical Research and Program of the Institute:

The finding that there is a significant incidence of cross reactivity between fetal antigens and tumor antigens in the guinea pig system provides further basis for the use of fetal antigens in immuno-diagnosis and possibly immuno-therapy. "Carcinoembryonic antigens" in patients with cancer of the GI tract,

cancer of the liver and elsewhere, are now specific enough to warrant further investigation when they are found in patients who may have an occult carcinoma, or possibly a recurrence of carcinoma after a resection. The possibility also exists that by heightening the immune response against antigens of this type, that prophylaxis against tumor growth may be achieved.

The finding in human patients of a high incidence of cross reactivity between patients with a histologically similar cancer (in this case, cancer of the lung and cancer of the cervix), suggests the presence of a common antigen in these cases. This lends further support to the hypothesis that certain human cancers may be produced by a virus which may leave antigens in different cases. The prospects are also raised for using allogeneic tissue for immuno-diagnosis or immunotherapy.

Proposed Course:

In ongoing studies in the Surgery Branch, the assessment of cutaneous hypersensitivity reactions against tumor cell antigens constitutes a valuable technique in patients who can be skin tested against their own tumor on multiple occasions as they are being immunized against that tumor in a concurrent immunotherapy program. The findings reported above will have to be applied in a wider group of animal and human tumor systems to further assess their general applicability.

Project Description:

Part III. Immunotherapy of Solid Tumors

Objective:

To boost the immunological defenses of a patient against his own cancer and thereby enable him to destroy minimum residual disease.

Methods Employed:

Approximately 40 patients with melanoma or sarcoma were treated in the Surgery Branch, National Cancer Institute, by Dr. Donald L. Morton four years ago in a program in which irradiated autochthonous or allogeneic tumor tissue was injected with BCG, and immune and clinical response measured. About two-thirds of these patients were melanoma patients and one-third were sarcoma patients. Among the melanoma patients, some patients who had only cutaneous disease received only intralesional BCG. Two patients with no residual cancer after regional node dissection received only intradermal BCG.

Major Findings:

Of 40 patients with melanoma and sarcoma treated with immunotherapy, six patients with melanoma are alive four years later and one patient with sarcoma is alive. Most of the six patients with melanoma had only cutaneous disease although the response to intralesional injection of BCG in patients with

satellitosis was quite dramatic in two patients. In one case, about 400 nodules were present as in transit metastases, and in another case, about 20 nodules were present. In both cases after inoculation of most but not all lesions, the satellitosis has disappeared and the patients remain free of active disease at the present time. In the other four surviving melanoma patients, the relative contribution of surgery and immunotherapy to their current survival is not determinable. The one patient with sarcoma who was treated with immunotherapy now has metastatic disease. There was an increase in antibody titer against melanoma and against sarcoma in patients with immunotherapy and autologous tumor cells.

Significance to Biomedical Research and Program of the Institute:

These studies have indicated that immunotherapy of melanoma and sarcoma with BCG is a feasible procedure. The toxicity of the BCG was mostly local ulceration and draining in the sites of BCG inoculation. No untoward effects were noted as a result of injection of irradiated tumor cells. In several patients, spiking fevers necessitated the discontinuance of BCG therapy, although it is possible that these were the patients getting the maximum immune response to the agent. The impact on survival in these patients has yet to be assessed. A control study will be necessary to measure this.

Proposed Course:

Protocols have been developed to assess the usefulness of immunotherapy in patients who have a bulky tumor that can be resected and used as a source for autochthonous cells. These cells will then be administered back to the patient with BCG. The cells will be treated with neuraminidase in order to uncover antigenic sites on the cell surfaces. It is hoped that by utilizing patients with minimal disease that the immunotherapeutic effects can be maximized. Additional joint studies are in progress with the Immunology Branch and the Chemotherapy Group to study melanoma patients who have a minimal amount of residual disease, but who stand a very high likelihood of succumbing from their disease because of the finding of positive regional lymph nodes. It is in patients with minimum amounts of disease that the maximum effects of immunotherapy will probably be seen, and it is to this group of patients that the main thrust of the program will be directed after toxicity studies have been fully evaluated.

Publications:

Melewicz, F.C., Wells, S.A., Christiansen, C., and Ketcham, A.S.: Delayed cutaneous hypersensitivity reactions to tumor extracts in patients with cervical carcinoma. Surg. Forum 23: 94-95, 1972.

Burdick, J.F., Cohen, A.M., Ketcham, A.S., and Wells, S.A., Jr.: An isotopic antiglobulin assay employing cell monolayers. Surg. Forum 23: 296-297, 1972.

Burdick, J.F., Cohen, A.M., and Wells, S.A., Jr.: A simplified isotopic antiglobulin assay for detection of tumor-cell antigen. J. Natl. Cancer Inst. 50: 285-289, 1973.

LeMevel, B.P., and Wells, S.A., Jr.: A microassay for the quantitation of cytotoxic antitumor antibody: Use of ^{125}I -iododeoxyuridine as a tumor cell label. J. Natl. Cancer Inst. 50: 803-806, 1973.

In Press:

Burdick, J.F., Wells, S.A., Cohen, A.M., and Ketcham, A.S.: Development of a simplified isotopic antiglobulin assay for study of tumor-specific antibody. J. Surg. Res.

Wells, S.A., Jr., Melewicz, F.C., Christiansen, C., and Ketcham, A.S.: Delayed cutaneous hypersensitivity reactions to membrane extracts of carcinomatous cells of the cervix uteri. Surg. Gynec. Obstet.

Wells, S.A., Burdick, J.F., Christiansen, C., Ketcham, A.S., and Adkins, M.D.: Demonstration of tumor associated delayed cutaneous hypersensitivity reactions in lung cancer patients and in patients with carcinoma of cervix. Natl. Cancer Inst. Monograph

Grant, J.P., Ladisch, S., and Wells, S.A.: Fetal antigens cross-reacting with tumor antigens in guinea pig sarcomas. Cancer

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3808

Title: Study of Host Factors Associated with Implantation and Growth of Cancer

Principal Investigators: S.A. Wells, Jr., M.D., and M.H. Cohen, M.D., Ph.D.

Section: Tumor Immunology Section

Lab/Branch: Surgery Branch

Goal: The purpose of this study is to develop in vivo and in vitro assays of immunity to correlate with and hopefully predict the clinical course in cancer patients undergoing standard therapy and immunotherapy.

Approach: Several assays have been used to monitor the immunological progression in mice, guinea pigs, and humans bearing cancer. These assays include the isotopic antiglobulin assay, the I¹²⁵-iododeoxyuridine cell label assay, and the micro-complement fixation assay. The results of these three in vitro assays of humoral immunity and the in vivo delayed hypersensitivity cutaneous reaction to measure cell mediated immunity were each correlated with the clinical course in animal or human systems.

Progress: The in vitro assays all revealed the presence of serum-mediated immunity in animals bearing methylcholanthrene-induced sarcomas. Using these assays, there seems to be more cross reaction between these tumors in mice than there was in guinea pigs. There was also cross reaction demonstrated by delayed cutaneous hypersensitivity reactions in patients with carcinoma of the lung and in patients with carcinoma of the uterine cervix. In each of these groups, over half of the patients who were skin tested against their own tumor or against another patient's tumor of the same histological type, demonstrated positive skin test reactions. Taken together, these studies indicate common antigenicity between different cases of the same histological type of tumor. These assays and others will be employed in cancer immunotherapy projects.

Significance to Cancer Research (NCP Objective 6, Approach 1): Through the development of these assays to monitor immunotherapy programs and through the possible benefit of these immunotherapy programs, it is hoped that these studies will be related to current efforts to develop a means to cure or retard cancers using immunologic methodology.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-3815
1. Surgery Branch
2. Tumor Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Transplantation of the Parathyroid Glands in Dogs

Previous Serial Number: Same

Principal Investigator: S. A. Wells, Jr., M.D.

Other Investigators: J. F. Burdick, M.D., A. S. Ketcham, M.D.,
C. Christiansen, B.S., M. Abe, M.D., L. Sherwood, M.D.,
B. G. Hattler, M.D., and R. C. Davis, M.D.

Cooperating Units: Endocrine Unit, Department of Medicine, Beth Israel
Hospital, Harvard Medical School, Boston, Massachusetts,
University of Arizona Medical Center, Tucson, Arizona,
and Memorial Hospital, Boston, Massachusetts

Man Years:

Total:	1
Professional:	1/2
Others:	1/2

Project Description:

Part I. Transplantation of the Parathyroid Glands in Dogs

Objectives:

To determine if transplanted autologous canine parathyroid glands are capable of function.

Methods Employed:

Three of the four parathyroid glands in dogs were surgically excised. Fragments of these glands were transplanted to the external oblique muscle. Ten days following grafting, the remaining in situ parathyroid gland was removed from its normal position in the neck. Partial thyroidectomy was also carried out to remove any accessory parathyroid tissue. At various intervals following this, the muscle beds containing grafted parathyroid glands were excised and the parathyroid hormone concentration of the transplants was determined by radioimmunoassay. Serum calcium determinations were done at intervals on all animals.

Nineteen dogs received their own sliced parathyroid glands as autografts. Parathyroid glands from seven dogs were transplanted as allografts to other animals. Serum calcium levels in both groups remained normal until the remaining in situ parathyroid was removed from the neck on day 10. By the 14th day, all seven allografted dogs were in tetany with a mean serum calcium of 4.6 mg/100 cc. The autografted group appeared healthy at this time and had a mean serum calcium level of 7.7 mg/100 cc. This was significantly higher ($p < 0.001$) than the level of the allograft group. Histologic examination of the autografted parathyroid glands removed on day 14 revealed normal appearing glands. The allografts were almost completely destroyed when examined on day 14. The parathyroid hormone content in the removed allografts was significantly lower than that in the removed autografts. Removal of autografts at 60 days was followed by prompt drop of serum calcium to 5.3 mg/100 cc and death of 11 of the 14 dogs within seven days. Histologically, these autografts appeared as normal parathyroid tissue with occasional pieces showing central fibrosis. The mean parathyroid hormone content of the glands removed from these dogs was significantly higher than that in the two week allografts but not significantly different from the PTH content of the two week autografts.

Significance to Biomedical Research and Program of the Institute:

During the course of surgery for certain thyroid cancers, as well as other primary cancers in the neck, it may be necessary to remove all parathyroid glands with adjacent tumor tissue. If allografts of parathyroid tissue could function sufficiently to maintain the host, this would considerably simplify the postoperative management of such tumor patients. Our observations indicate that parathyroid gland allografts in the dog are rejected in a manner similar to other allogeneic tissues. The finding that autografts function sufficiently well to maintain a normal serum calcium concentration in dogs suggests that following inadvertent excision of parathyroid tissue during thyroid and other neck surgery, this tissue be transplanted as small pieces within a muscle bed in an attempt to maintain normal postoperative parathyroid function.

Proposed Course:

Further investigations will be carried out to develop the best techniques of parathyroid transplantation to ensure optimum growth of these transplants.

Publications:

Wells, S.A., Jr., Burdick, J.F., Ketcham, A.S., Christiansen, C., Abe, M., and Sherwood, L.: Transplantation of the parathyroid glands in dogs. Biochemical, histological, and radioimmunoassay proof of function. Transplantation 15:179-182, 1973.

Wells, S.A., Burdick, J.F., Christiansen, C., Abe, M., Sherwood, L., Hattler, B.G., and Davis, R.C.: Long-term survival of dogs transplanted with parathyroid glands as autografts and as allografts in immunosuppressed hosts. Transplantation Proceedings 5(1):769-772, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3815

Title: Transplantation of the Parathyroid Glands in Dogs

Principal Investigator: S. A. Wells, Jr., M.D.

Section: Tumor Immunology Section

Lab/Branch: Surgery Branch

Goal: To develop methods for successful transplantation of functioning parathyroid tissue in dogs.

Approach: Excision of three of the four parathyroid glands was carried out in dogs. Fragments of these glands were implanted within the external oblique muscle. Ten days following grafting the remaining in situ parathyroid gland of each dog was removed from the neck. Partial thyroidectomy was also carried out to remove potential accessory parathyroid tissue. Nineteen dogs received their own parathyroid glands as autografts. Six dogs received parathyroid allografts. The grafted glands were excised at intervals for histologic study and determinations of parathormone content. Serum calcium determinations were monitored in all animals.

Progress: Serum calcium in both groups of animals remained normal until the remaining in situ parathyroid gland was removed from the neck on day 10. By the 14th day, all seven allografted dogs were in tetany with the depressed calcium levels. At this time, the healthy appearing autografted group had calcium levels which were somewhat depressed as compared to normals, but were significantly higher than the allografted group. Excised autografted parathyroids appeared perfectly normal histologically on day 14, while the allografts were almost completely destroyed by a process characteristic of acute graft rejection. The parathormone content in excised allografts was significantly lower than the parathormone content in the removed autografts. Data demonstrate that allografted parathyroid glands do not function sufficiently to maintain the host and are rejected in a manner similar to other allogeneic tissues. Autografts transplanted as small pieces within a muscle bed function sufficiently well to maintain normal serum calcium concentrations.

Significance for Cancer Research (NCP Objective 7, Approach 1): These studies have implications for rehabilitation of those patients requiring excision of parathyroid tissue for the treatment of thyroid cancer or other tumors of the neck. If normal parathyroid tissue can be harvested from such patients or from patients undergoing inadvertent excision of parathyroid tissue during surgery, attempts should be made to autograft this tissue within a muscular bed to prevent postoperative hypoparathyroidism.

Level of Effort: Professional Man-Years: 1/2

SUMMARY REPORT
RADIATION BRANCH
NATIONAL CANCER INSTITUTE

July 1, 1972 through June 30, 1973

Continued investigation of multi-system therapy (MST) has remained the dominant theme of the research program during the past year. The highly successful program on Ewing's sarcoma has proven to be an invaluable "stalking horse" for developing a general rationale of combined therapeutics. A programmatic approach to integrated therapy or MST has gradually evolved and systematic description of methodology will shortly be possible for extrapolation to other forms of cancer. Fundamentally, the essential ingredients for MST consist of careful documentation of the natural history of any cancer, development of methodology for accurately discerning the location of any patient within the overall spectrum for that disease, determining the "success rate" for single treatment modalities, analyzing the mechanisms of failure pertinent to each modality, and finally selecting treatment approaches which complement by virtue of negating the weakness of one modality via the strength of another modality.

The adoption of Ewing's sarcoma as a model system to investigate combined therapy from a conceptual standpoint has been a fortunate choice as this neoplasm is more comparable to the most common forms of cancer than the leukemia-lymphoma axis of cancer in man in terms of biology and "management problems." Sixty patients with Ewing's sarcoma have been intensively studied to date and many lessons have been learned with respect to MST, not only in theory but also in practice. Perhaps the strongest witness to the potential effectiveness of integrated therapeutic approaches is the dramatic increase in survival which has been observed in this clinical program. One-third of patients presenting with metastatic disease have survived three years and nearly one-half of patients with localized involvement on admission are alive at three years, the majority of these latter having no evidence of residual cancer.

Concepts and techniques learned from the Ewing's study have been programmed for extension to cancer of the lung as described in last year's annual report. Unfortunately, the lung cancer study has been seriously hampered by limitations in facilities to support such a program. This pertains not only to the need for additional research beds but also to increased support in terms of nursing care and diagnostic facilities. These constraints have restricted the admission of lung cancer patients to a small number although the experience to date has served to familiarize the staff with the pertinent difficulties faced in developing an effective therapy for lung cancer. Hopefully, the next year will witness an appreciably accelerated effort directed toward this most important national health problem.

While closed to accession of new patients, the Hodgkin's disease study has continued to yield important information. Survival rates for patients with

SUMMARY REPORT (continued)

Stage I and II involvement remain the highest reported in the world for the optimal test group, the six-year survival rate being in excess of ninety percent. The long-term observation now available has permitted meaningful retrospective analysis of many subjects, including the reliability of the diagnostic procedures commonly employed, the comparative efficacy of various therapeutic techniques, complication rates associated with different radiotherapy or combined radiotherapy-chemotherapy approaches, and problems related to rehabilitation. Overall, this study of Hodgkin's disease and its management has illustrated the comprehensive depth with which clinical research can be pursued at unique institutions such as the National Cancer Institute.

The study of other malignant lymphomas in collaboration with the Medicine Branch of the National Cancer Institute has been extremely active in terms of accession rate during the past year. Carefully designed diagnostic and therapeutic programs have been initiated and will unquestionably generate results commanding as wide attention as the program on Hodgkin's disease. Already the efficacy of total body irradiation using fractionated dosage schedules has become established and is gaining increased application throughout the country. Essential information regarding the natural history of the malignant lymphomas is rapidly being acquired and the multidisciplinary approach which earmarks the study overall is unparalleled in cancer therapeutics. A peripherally related effort over the past decade has been the study on chronic lymphocytic leukemia and nearly one hundred patients have been seen in the Radiation Branch. Data on these latter is currently being compiled for computerization and analysis will hopefully shed some urgently needed light on the pathogenesis and management of this often neglected and extremely enigmatic form of leukemia.

A major event in the research program of the Radiation Branch during the past year has been the development of a computer program for the storage, continuous editing, and retrieval of clinical data. At present, approximately 300 patients have their clinical records computerized, thereby permitting retrieval of information heretofore impossible for research patients treated at the Clinical Center. In addition to facilitating usual determinations of treatment efficiency such as remission rates and survival rates, data can now be readily retrieved which is concerned with epidemiology, the natural history of disease, methodology of diagnostic evaluation, immunologic status, comparative types of treatment and the mechanisms of failure for the individual treatment approaches. This has been a time-consuming and painstaking effort but will clearly expand and improve the quality of our research activities.

The escalation of clinical activity has ever continued with the number of patient visits reaching an annual rate of 7500 during the past year. This work load approaches our logistic capacity for outpatient programs and the initiation of new studies will be contingent upon the phasing out of current programs as objectives are achieved and upon any additional assignment of research beds to the Radiation Branch. The past year has also witnessed

SUMMARY REPORT (continued)

a significant upgrading of treatment facilities as the consequence of the research linear accelerator becoming operational and the installation of a second linear accelerator as a substitute unit. Finally, the Radiation Branch has continued to provide extensive support to other program areas of the National Cancer Institute in terms of advice, thousands of non-clinical radiation exposures, and several hundred patient consultations during the year.

Serial No. NCI-650 (c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Service Radiation Therapy

Previous Serial Number: Same

Principal Investigator: Thomas C. Pomeroy, M.D.

Other Investigators: Kent B. Lamoureux, M.D.; Larry E. Kun, M.D.;
Frederick L. Faw, M.S.; Dwight W. Glenn, M.S.;
Marilyn K. Glover, R.N.; Sandra K. Johnson, R.N.;
Margaret R. Gable, R.T.

Cooperating Units: None

Man Years:

Total: 4/2
Professional: 3/2
Other: 1/2

Project Description:

Objectives: To provide expert radiotherapy consultation and treatment for Clinical Center patients admitted to services other than the Radiation Branch of the National Cancer Institute.

Patient Material: During the twelve month period preceding this report, 194 patients were formally seen in consultation. Advice and management of many additional patients was provided on an emergency or "ad hoc" basis including over 130 logged telephone consultations for advice on treatment or general information.

Methods Employed: The majority of patients were irradiated with the recently installed 6- and 8-MeV linear accelerators or Cobalt teletherapy following appropriate evaluation and treatment planning as indicated.

Major Findings: Aside from results on research patients as described in project reports from other institutes, the response to radiation therapy has been consistent with that reported from other major centers.

Proposed Course: To continue.

Honors and Awards: None

Publications: None

Serial No. NCI-958(c)
1. Radiation Branch
2. Radiation Therapy & Physics
Sections
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Treatment of Hodgkin's Disease

Previous Serial Number: Same

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Dwight W. Glenn, M.S. and Frederick L. Faw, M.S.

Cooperating Units: Medicine Branch, NCI

Man Years:

Total: 4/2
Professional 3/2

Project Description:

Objectives: This study is directed toward improving the effectiveness of therapy for Hodgkin's disease, using radiation therapy alone and in combination with chemotherapy.

Patient Material: Two hundred patients with previously untreated Hodgkin's disease have been entered into prospective protocol studies since the initiation of the program in January 1965.

Methods Employed: The initial approach was to examine various radio-therapeutic approaches used alone for the treatment of Hodgkin's disease in a controlled study to determine (1) which method(s) yielded optimal results and (2) what type of patient could not be adequately managed with radiotherapy alone. Upon identification of subsets of patients at high risk of failing to be cured with radiotherapy alone as primary management, studies were initiated to investigate the combined use of radiotherapy and chemotherapy on a selective basis.

Major Findings: The findings have been detailed in numerous publications and have permitted conclusions as to the accuracy of diagnostic tests in determining disease extent, the need for prophylactic irradiation according to clinical presentation, the potential for long-term survival and potential cure in various types of patients, and the defining of which patients require a combined approach with radiation and chemotherapy for optimal results.

Proposed Course: This study has now been closed to accession of additional patients but long-term observation will be continued on surviving patients for documentation of important features such as delayed complications secondary to treatment, incidence of second malignancies, etc.

Honors and Awards: None

Publications:

ARTICLES PUBLISHED IN A BOOK

Johnson, R.E.: Total Nodal Irradiation of Hodgkin's Disease: Technical and Clinical Aspects. (In) Textbook of Radiotherapy, Edit. by G. Fletcher.

ARTICLES PUBLISHED IN A PERIODICAL

Wilson, J.F., Marsa, G.W., And Johnson, R.E.: Herpes Zoster in Hodgkin's Disease: Clinical, Histologic, and Immunologic Correlations. Cancer 29: 461, 1972.

Johnson, R.E.: Radiation Therapy in Hodgkin's Disease. J.A.M.A. 223: 59, 1973.

Kagan, A.R., Hafermann, M., Hamilton, M., Pierce, R., and Johnson, R.E.: Etiology, Diagnosis, and Management of Pericardial Effusion after Irradiation. Radiologia, Clinica, et Biologica 41: 171, 1972.

Glenn, D.W., and Johnson, R.E.: Gonadal Shielding During Cobalt Teletherapy. Radiology 104: 214, 1972.

Arseneau, J.D., Sponzo, R.W., Schnipper, L.E., Bonner, H., Young, R.C., Canellos, G.P., Johnson, R.E., and DeVita, V.T.: Nonlymphomatous Malignancies Complicating Hodgkin's Disease: Possible Association with Intensive Therapy. New Engl. J. Med. 287: 1119, 1972(Nov 30).

Johnson, R.E.: Radiation Management of the Lymphomas. Proceedings of the Seventh National Cancer Conference. Cancer(In Press)

Young, R.E., DeVita, V.T., and Johnson, R.E.: Hodgkin's Disease in Childhood, Blood(In Press)

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-958 (c)

Title: The Treatment of Hodgkin's Disease

Principal Investigator: Ralph E. Johnson, M.D., Dwight W. Glenn, M.S.

Section: Radiation Therapy Service and Radiation Physics Section

Lab/Branch: Radiation Branch

Goal: To improve the effectiveness of therapy for Hodgkin's disease, using radiation therapy alone and in combination with other modalities.

Approach: The initial approach was to examine various radiotherapeutic approaches used alone for the management of Hodgkin's disease for the purpose of determining (1) which method yielded optimal results and (2) what types of patients could or could not be adequately managed with irradiation as the only form of treatment. The identification of certain patient populations having a high risk of failure with radiotherapy alone led to the current approach of combining systemic chemotherapy with local radiotherapy.

Progress: The radiotherapy studies have yielded the highest described survival rates in the world for patients with clinically localized Hodgkin's disease. Selected groups of patients with apparently early involvement in addition to those patients with more advanced disease are currently being accessioned into combined therapy protocols involving radiation therapy and chemotherapy used sequentially. A carefully designed prospective study into which 134 patients were entered demonstrated the value of prophylactic irradiation for the first time in a properly controlled clinical trial and the six year survival rate for the extensively irradiated group of Stage I and II patients overall exceeds 90%. In addition, technical aspects of radiotherapy have received intensive examination, resulting in the development of numerous new technical approaches and one device with U.S. patent. Significance for Cancer Research (NCP Objective 6 Approach 6)

This program has direct relationship to the cancer effort in terms of improving the cure rate for types of cancer for which, to date, there are no means of prevention or earlier detection.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-963
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Effects of Immunologic Manipulation on Radiation
Carcinogenesis and Radiation Response of Tumors

Previous Serial Number: Same

Principal Investigator: Thomas C. Pomeroy, M.D.

Other Investigators: Walter G. Hardy

Cooperating Units: None

Man Years:

Total:	5/4
Professional:	1/4
Other:	4/4

Project Description:

Objectives: To study the effects of immunologic manipulation on the incidence and time of appearance of radiation induced tumors and the effect of age at the time of irradiation, and the effects of immunologic manipulation on the radiation response of tumors.

Methods Employed: Total body radiation of mice with cyclic immunosuppression and in vitro radiation of mouse kidney cells with reintroduction of cells intravenously in cyclically immunosuppressed mice are the methods utilized to evaluate the alterations in types of tumors and time of development of radiation induced benign and malignant neoplasms. Parallel control groups are expected to reveal differences in "spontaneous" tumor production.

Previous studies have shown variations in tumor responsiveness to radiation by non-specific immunologic suppression and enhancement. Presently, histopathologic description and correlation of the attendant tissue reactions is being continued.

Major Findings: Immunosuppression delays onset of lymphomas in both control and irradiated mice. In contrast, tumor development other than lymphomas is accelerated in both control and irradiated mice with a greater variety of tumors in the irradiated mice. Despite increased tumor production, cyclic immunosuppression has increased survival rates significantly in both control and irradiated mice. A few adenocarcinomas

have developed after in vitro radiation of kidney cells. Histopathologic changes resemble those of graft rejection where associated with either immunologic enhancement or recovery from immunologic suppression.

Continued accumulation of data have confirmed the preliminary results reported. The radiation portion was repeated to obtain further data on the early development of lymphomas and is near completion. No data is available as yet on comparative age differences in immunologic factors involved in radiation carcinogenesis. The findings may have application in further elucidation of the differences in initiation and development of lymphomas as contrasted to epithelial tumors and soft tissue sarcomas. The role of steroids as a mitotic inhibitor active in delaying the aging process as well as the time of development of lymphomas may simply be overbalanced by the greater importance of immune mechanisms in controlling evolution of the epithelial and connective tissue tumors.

A comparative study using chemical carcinogenesis has been initiated but no results are available.

Proposed Course: Complete analysis of carcinogenesis studies. Continue studies on in vitro radiation and re-innoculation of isologous kidney cells.

Honors and Awards: None

Publications:

Thomas C. Pomeroy, M.D., and Walter G. Hardy: Enhancement of Radiation Carcinogenesis in Mice by Cyclic Immunosuppression With Cortisone Acetate. Proc. Amer. Assoc. Cancer Res. 13:25, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-963 (c)

Title: Effects of Immunologic Manipulation on Radiation Carcinogenesis and Radiation Response of Tumors

Principal Investigator: Thomas C. Pomeroy, M.D.

Section: Radiation Therapy Service

Lab/Branch: Radiation Branch

Goal: Determine the effects of immunologic suppression on radiation carcinogenesis and immunologic suppression and enhancement on radiation response of tumors.

Approach: Total body irradiation (380 rads) is followed by weekly injections of Cortisone Acetate for the life span of the Swiss-Webster mouse. Time of occurrence and type of tumor production is analyzed in comparison to radiation alone and control mice and mice immunosuppressed only. This same approach will be evaluated in mice of varying ages 3 months, 9 months, and 15 months at the time of irradiation.

A parallel study will be conducted with I.V. 7, 12 DMBA as the tumor initiator.

Isologous mouse kidney cells irradiated in vitro will be re-introduced in the mouse with concomitant immunosuppression.

Histologic evaluation of radiation response patterns will be compared with graft rejection patterns.

Progress: Immunosuppression significantly increases the production of and advances the time of appearance of epithelial and connective tissue tumors as contrasted to the decrease of, and/or delay in appearance of lymphomas.

This demonstrates a basic difference in the progression of radiation induced lymphomas and other tumors in relation to immunologic mechanisms with immune surveillance possibly occupying a greater role in the non-lymphomatous diseases.

Immunologic suppression and enhancement by non-specific means produces variations in radiation response which correlate with changes in histologic patterns similar to those seen in metastatic tumor or graft rejections. Significance for Cancer Research (NCP Objective 1 Approach 3)

Level of Effort: Professional Man-Years: 1/4

Serial No. NCI-964 (c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Study of Cancer of the Upper Air Passages and the Evaluation of Combined Treatment Approaches.

Previous Serial Number: Same

Principal Investigator: Thomas C. Pomeroy, M.D.

Other Investigators: Walter G. Hardy; Ralph E. Johnson, M.D.;
Adelbert Evans, Ph.D.; Robert K. Oldham, M.D.

Cooperating Units: Laboratory of Cell Biology, Division of Cancer Biology and Diagnosis; and the Armed Forces Radiobiological Research Institute.

Man Years:

Total: 5/4
Professional: 2/4
Other: 3/4

Project Description:

Objectives: To evaluate the comparative toxicity and usefulness of various chemotherapeutic agents as adjuvants to radiation therapy in patients with malignant tumors of the mouth and throat.

Laboratory investigations with animal tumor systems will attempt to elucidate additive or synergistic responses and/or toxicity of various combinations of modalities and sequences of administration.

Patient Material: Patients with post-surgical and post-irradiation recurrences and primary advanced inoperable squamous cell carcinomas of mouth and throat are accepted for study.

Methods Employed: (1) Post-surgical and post-radiation recurrences of and advanced primary squamous cell carcinomas of mouth and throat are evaluated for toxicity and tumor response to selected agents for which evidence exists suggesting a response for this type of malignancy (e.g. Bleomycin, Methotrexate, and Adriamycin). (2) Active drugs and combinations of active drugs will be added to radiation therapy in post-surgical recurrent and advanced inoperable epidermoid carcinomas of the mouth and throat. (3) Laboratory studies combine radiation of solid implants with systemic chemotherapy in varied sequences and time-dose relationships,

and evaluation of various combined schedules of Bleomycin, Adriamycin, and Methotrexate in the L 1210 Leukemia and other model systems.

Major Findings:

A. Clinical Findings

1) Bleomycin alone in 19 cases has shown greater than 50 percent regression in six cases and 25-50 percent regression in an additional seven. The remaining six cases showed only minimal response.

2) Bleomycin combined with radiation therapy in 14 Stage III and IV lesions showed complete clinical clearance of the primary in ten and greater than 90 percent in two additional cases. Node regression was complete in eight and greater than 50 percent in an additional five. Only one patient with complete clinical regression has relapsed locally; however, six have developed disseminated disease and two more have suspected lung or liver metastasis. The results indicate a possible role for Bleomycin combined with radiation therapy in increasing local control rates in moderately advanced disease (Stage II and some Stage III) as despite greatly augmented mucosal reactions, the radiation dose in most instances has been low and subsequent fibrosis and mucosal changes have been minimal compared to patients treated to higher doses with radiation alone to such large volumes.

More aggressive chemotherapy will be required to salvage more of the advanced cases. MTX and Adriamycin have produced additive local response in refractory situations.

More encouraging has been regression of greater than 50 percent in multiple lung metastases by a combination of ADM and VC after pulmonary toxicity of Bleomycin and systemic intolerance to MTX. Another patient may demonstrate the ability of drugs to decrease numbers of subsequent appearing clinical metastasis. This patient has survived over one year following resection of a solitary lung nodule.

The major drawback to chemotherapy has been the poor tolerance of this type of patient to drugs, particularly in the elderly. These patients almost universally have a history of alcoholism with attendant accumulated nutritional damage, particularly of the liver and gastrointestinal tract.

B. Laboratory Findings

1) L 1210 leukemia in DBA mice has proven to be a poor model for evaluation of Bleomycin combined with other drugs. This appears related to increased susceptibility to lung infections in standard housing situations with this breed. Erratic results of toxicity in establishing optimum dosage of MTX has also been observed in addition

to the greater toxicity of Bleomycin with its high lung concentration.

Combined studies of drugs have been postponed until a suitable transplantable squamous cell tumor model is obtained (none available at the NCI at present.)

2. Bleomycin was observed to produce tumors at the site of injection in 3/15 mice held for long term toxicity observation after daily subcutaneous injections for six weeks. The latent period of tumor development was three to four months. Two of the three tumors were sarcomas and one has been transplanted over thirty generations in Swiss Webster mice and test transplanted to DBA mice.

Considerable effort has gone into a randomized study with both S.C. and I.M. inoculation with saline injected controls. Injections were given three times weekly for ten weeks when beginning suggestion of drug toxicity appeared. To date, ten sarcomas, 8/50 S.C., 2/50 I.M., have been produced and identified with several transplanted in multiple generations. All have been similar histologically and appear to be fibrosarcomas with some areas of synovial or vascular differentiation.

In addition, many other tumors have appeared in the Bleomycin groups only (lymphomas, pulmonary adenomas, and ovarian tumors). These tumors occur spontaneously in this strain of mouse but at a later age. Also of interest has been development of a granulomatous infection which can be transmitted by I.P. inoculation to normal mice. This usually involved salivary and lacrimal glands, lymph nodes, and kidneys. Histologically, the granulomas contain clumps of material resembling fine hyphae (Bleomycin is derived from *Streptomyces Verticillius*); however, attempts to culture fresh material in standard media for fungus has not been successful.

Proposed Course:

- 1) Continue clinical studies with additional evaluation of prognostic indicators (Glycoprotein profiles; T/B lymphocyte ratios; micro-cytotoxicity assays; and macrophage inhibition factor determination), all recently initiated.
- 2) Complete observations and publish results of Bleomycin-Sarcoma study.
- 3) Continue combination drug evaluation in a more suitable murine transplantable squamous cell carcinoma (available from Dr. Dunning, Papanicolaou Institute).

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-964 (c)

Title: The Study of Cancer of the Upper Air Passages and the Evaluation of Combined Treatment Approaches

Principal Investigator: Thomas C. Pomeroy, M.D.

Section: Radiation Therapy Service

Lab/Branch: Radiation Branch

Goal: Determine the role of adjunctive chemotherapy to the radiation management of advanced oropharyngeal cancer in man. Evaluate toxicity and response of tumors to combination drug therapies in murine tumor models.

Approach: Patients with Stage III and IV or post-surgical recurrent oropharyngeal cancer will be admitted for combined therapy with radiation and chemotherapy. Initially, a response rate and regression will be determined for Bleomycin with radiation initiated at maximum regression or at a maximum of six weeks. Other drugs will be evaluated where incomplete response or relapse occurs.

Murine tumors will be used for determination of response and toxicity of drugs reported to have useful response rates in clinical situations. Particular emphasis will be on combinations.

Progress: Bleomycin has produced greater than 50 percent regression in 6/19 patients. An additional 7/19 have had 25-50% regression. In 14 of these patients evaluable, addition of radiation therapy has provided complete primary clearance in 10, and greater than 90% regression in 2 additional. Complete regression of node masses occurred in 8/14.

Animal studies have not added significantly to the clinical approach to date. L1210 leukemia in DBA mice has proved a poor model due to susceptibility of the strain to respiratory infections as well as bleomycin lung toxicity.

More extensive studies are in progress on sarcoma production from repeated local injections of Bleomycin after observation of two sarcomas developing with a latent period of 3-4 months after last injections.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Progress (cont.): To date, ten additional sarcomas have been produced with S.C. or I.M. injections.

Level of Effort: Professional Man-Years: 2/4

Serial No. NCI-965 (c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Study of Hand-Schuller-Christian Disease and the Role
for Radiation Therapy of the Hypothalamic-Pituitary Lesion

Previous Serial Number: Same

Principal Investigator: Thomas C. Pomeroy, M.D.

Other Investigators: Mortimer Lipsett, M.D., and Griff Ross, M.D.

Cooperating Units: National Institute of Child Health and Development-
Reproduction Research

Man Years:

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: To determine the results of radiation therapy directed to the hypothalamic-pituitary region on the growth hormone production and the severity and progression of diabetes insipidus in patients with Hand-Schuller-Christian disease.

Patient Material: Fifteen patients in the pre-pubertal age range with biopsy proven Hand-Schuller-Christian disease with diabetes insipidus, with or without clinical growth retardation, but without generalized disease will be acceded to the study.

Methods Employed: Extensive clinical and laboratory evaluation of each patient will precede radiation therapy. This will include growth hormone assays and water loading tests. Radiation therapy (1200 rads in two weeks) will be followed by serial determinations of growth hormone production and evaluation of any changes in growth patterns and severity of diabetes insipidus.

Major Findings: Results in the first case treated, though preliminary, have shown an increase in the growth hormone level after radiation, but without increase in rate of growth. A second case has shown increased growth rate but no significant change in growth hormone level.

Proposed Course: Project to be discontinued as there has been no additional patient acquisition. If additional patients are added, the study will continue under NCI-650 (c) (Service Radiation Therapy)

Honors and Awards: None

Publications: None

Serial No. NCI-966(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Tumor Diagnosis with Microwaves

Previous Serial Number: Same

Principal Investigator: Dwight W. Glenn, M.S.

Other Investigators: Thomas C. Pomeroy, M.D.

Cooperating Units: None

Man Years:

Total 1/30

Professional: 1/30

Project Description:

Objectives: To consider the feasibility of using microwave techniques in tumor diagnosis and to assist the contractor in obtaining funds for the project.

Methods: A preliminary literature search indicated support appeared desirable. Assistance was given to the proposed contractor (Kearfott Division of Singer Company) in submitting a proposal to the Breast Task Force for funding.

Proposed Course: The Diagnostic Subcommittee (BTF) voted to shelve this project for one year. Limited consultation with Kearfott will be maintained in the interim.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-966(c)

Title: Tumor Diagnosis with Microwaves

Principal Investigator: D. W. Glenn, T. C. Pomeroy

Section:

Lab/Branch: Radiation Branch

Goal: To consider the feasibility of using microwave techniques in tumor diagnosis and to assist the contractor in obtaining funds for the project.

Approach: A preliminary literature search indicated support appeared desirable. Assistance was given to the proposed contractor (Kearfott Division of Singer Company) in submitting a proposal to the Breast Task Force for funding.

Progress: The Diagnostic Subcommittee (BTF) voted to shelve this project for one year. Limited consultation with Kearfott will be maintained in the interim.

Significance for Cancer Research (NCP Objective 5B Approach 4)

Early detection of breast cancer thru an acceptable mass screening program would appear to be feasible with the proposed project.

Level of Effort: Professional Man-Years: 1/30

Serial No. NCI967
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Measurement of "W" for 14 Mev Neutrons

Previous Serial Number: Same

Principal Investigator: Dwight W. Glenn, M.S.

Other Investigators: Nathan Coffey

Cooperating Units: None

Man Years:

Total: 1 1/4
Professional: 1/2

Project Description:

Objectives: To obtain a value of "W" for neutron beams to enable the Bragg-Gray theory to apply to ionization measurements, i.e., relate ionization in a material to the absorbed dose in that material.

Methods: The absorbed dose will be measured in a tissue-equivalent material by determining directly, the amount of energy deposited. The amount of energy deposited will be related to the amount of ionization measured in a similar material. (Calorimeter compared to ionization in a tissue-equivalent gas-filled ionization chamber). The systems will be irradiated with cobalt irradiation to determine some basic characteristics of the system whereas the final measurements will be done with neutron beams.

Proposed Course: The fabrication of the two systems has been completed as well as the associated circuitry. Information has been obtained with the ionization chamber that indicates gas flow alters the amount of ionization observed that is not attributable to changes in pressure. Stability tests are currently being conducted with the calorimeter. Data collection will be initiated using cobalt irradiation. If successful, neutron data collection will follow.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 967

Title: Measurement of "W" for 14 Mev Neutrons

Principal Investigator: D. W. Glenn

Section: Radiation Physics Section

Lab/Branch: Radiation Branch

Goal: To obtain a value of "W" for neutron beams to enable the Bragg-Gray theory to apply to ionization measurements, i.e., relate ionization in a material to the absorbed dose in that material.

Approach: The absorbed dose will be measured in a tissue-equivalent material by determining directly, the amount of energy deposited. The amount of energy deposited will be related to the amount of ionization measured in a similar material. (Calorimeter compared to ionization in a tissue-equivalent gas-filled ionization chamber). The systems will be irradiated with cobalt irradiation to determine some basic characteristics of the system whereas the final measurements will be done with neutron beams.

Progress: The fabrication of the two systems has been completed as well as the associated circuitry. Information has been obtained with the ionization chamber that indicates gas flow alters the amount of ionization observed that is not attributable to changes in pressure. Stability tests are currently being conducted with the calorimeter.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Will enhance dosimetry for neutron irradiation studies.

Level of Effort: Professional Man-Years: 1/2

Serial No, NCI-968(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Gonadal Shielding

Previous Serial Number: Same

Principal Investigator: Dwight W. Glenn, M.S.

Other Investigators: Ralph E. Johnson, M.D.

Cooperating Units: None

Man Years:

Total: 1/30

Professional: 1/30

Project Description:

Objectives: To determine an efficient method of shielding the male gonads during radiotherapy of the pelvic region.

Methods: Thermoluminescent and ionometric methods of dosimetry utilized in phantoms and on patients undergoing radiotherapy while varying shielding techniques.

Major Findings: A method was arrived at whereby the gonadal dose was reduced to approximately 50% from that received by other shielding methods. The application of this technique will be verified for higher energies, i.e., 6 Mv and 8 Mv photon beams.

Honors and Awards: None

Publications:

Glenn, D.W and Johnson, R.E.: Male Gonadal Shielding During Cobalt Teletherapy. Radiology 104:214, July 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 968(c)

Title: Gonadal Shielding

Principal Investigator: D. W. Glenn, R. E. Johnson

Section:

Lab/Branch: Radiation Branch

Goal: To determine an efficient method of shielding the male gonads during radiotherapy of the pelvic region.

Approach: Thermoluminescent and ionometric methods of dosimetry utilized in phantoms and on patients undergoing radiotherapy while varying shielding techniques.

Progress: A method was arrived at whereby the gonadal dose was reduced to approximately 50% from that received by other shielding methods. The information was published in Radiology, July 1972, for cobalt teletherapy. The application of this technique will be verified for higher energies, i.e., 6 Mv and 8 Mv photon beams.

Significance for Cancer Research (NCP Objective 6 Approach 1)

A more effective treatment plan was obtained that reduces the radiation dose to the male gonads to a more acceptable level.

Level of Effort: Professional Man-Years: 1/30

Serial No. NCI-969(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Large Field Dosimetry

Previous Serial Number: Same

Principal Investigator: Frederick L. Faw, M.S.

Other Investigators: Dwight W. Glenn, M.S.

Cooperating Units: None

Man Years:

Total: 1/10
Professional: 1/10

Project Description:

Objectives: To determine an accurate method of assessing the radiation dosimetry in fields that are much larger than the irradiated object.

Methods: Utilizing thermoluminescent and ionometric dosimetry systems, radiation doses in different size phantoms are compared to varying field sizes (larger than the phantom) and photon energies.

Major Findings: Work is continuing for 6 Mv and 8 Mv photon beams. For cobalt irradiation, it was found that the size of the phantom is the basis for dose assessment regardless of how much larger the irradiation beam.

Honors and Awards: None

Publications:

Faw, F.L. and Glenn, D.W.: Large Field Dosimetry. Proceedings of International Medical Physics Conference, Goteburg, Sweden, July, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 969(c)
Title: Large Field Dosimetry

Principal Investigator: F. L. Faw, D. W. Glenn
Section: Radiation Physics Section
Lab/Branch: Radiation Branch
Goal: To determine an accurate method of assessing the radiation dosimetry in fields that are much larger than the irradiated object.

Approach: Utilizing thermoluminescent and ionometric dosimetry systems, radiation doses in different size phantoms are compared to varying field sizes (larger than the phantom) and photon energies.

Progress: Data was accumulated for cobalt teletherapy and presented at the International Medical Physics Conference in Goteburg, Sweden, in July 1972. Work is continuing for 6 Mv and 8 Mv photon beams. For cobalt irradiation, it was found that the size of the phantom is the basis for dose assessment regardless of how much larger the irradiation beam.

Significance for Cancer Research (NCP Objective 6 Approach 1)

A more accurate means of determining radiation doses to patients undergoing whole-body treatment and extremity irradiation is available.

Level of Effort: Professional Man-Years: 1/10

Serial No. NCI-970(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Patient Positioning Television System

Previous Serial Number: Same

Principal Investigator: Albert P. Wheatley

Other Investigators: Dwight W. Glenn, M.S. and Ralph E. Johnson, M.D.

Cooperating Units: None

Man Years:

Total: 1/10

Professional: 1/30

Project Description:

Objectives : To devise a system whereby patient treatment positioning is accurately and easily reproduced from day to day.

Methods: Photography and television systems are combined into a single system whereby the original treatment position image from photography is simultaneously viewed with later treatment positions from television cameras which allow the therapy technician to adjust the patient to conform to the original treatment position.

Major Findings: Preliminary studies with curved image receptors indicate unacceptable errors in the peripheral aspect; a flat faced monitor was purchased to eliminate this error - current studies are in progress to determine the difference.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 970(c)

Title: Patient Positioning Television System

Principal Investigator: A. P. Wheatley

Section: Radiation Physics Section

Lab/Branch: Radiation Branch

Goal: To devise a system whereby patient treatment positioning is accurately and easily reproduced from day to day.

Approach: Photography and television systems are combined into a single system whereby the original treatment position image from photography is simultaneously viewed with later treatment positions from television cameras which allow the therapy technician to adjust the patient to conform to the original treatment position.

Progress: Preliminary studies with curved image receptors indicate unacceptable errors in the peripheral aspect; a flat faced monitor was purchased to eliminate this error - current studies are in progress to determine the difference.

Significance for Cancer Research (NCP Objective 6 Approach 1)

A successful system will allow radiation treatments to be given with less error due to positioning the patient in the beam.

Level of Effort: Professional Man-Years: 1/30

Serial No. NCI-975(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Study of Tumor Biology and Diagnostic Evaluation in Lung Cancer

Previous Serial Number: None

Principal Investigator: Kent B. Lamoureux, M.D.

Other Investigators: Ralph E. Johnson, M.D.

Cooperating Units: NHLI, Clinical Center (Diagnostic Radiology and Nuclear Medicine), and Laboratory of Pathology, NCI

Man Years:

Total: 3/2
Professional: 2/2

Project Description:

Objectives: The purpose of this study is to improve understanding of the natural history of lung cancer by means of extensive diagnostic evaluation at the time of diagnosis and serially throughout the course of disease.

Patient Material: Approximately 25 patients have been entered into this program.

Methods Employed: Exhaustive diagnostic evaluation of each patient has been undertaken using both standard and investigational techniques and these studies have been repeated and correlated with clinical findings and developments for estimation of reliability.

Major Findings: An insufficient number of patients have been studied to permit conclusions which vary substantially from the commonly accepted ideas regarding the biologic behaviour and natural history of lung cancer.

Proposed Course: Accession of patients into this program will continue.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-975(c)

Title: Study of Tumor Biology and Diagnostic Evaluation in Cancer of the Lung

Principal Investigator: Kent B. Lamoureux, M.D. and Ralph E. Johnson, M.D.

Section: Radiation Therapy Service

Lab/Branch: Radiation Branch

Goal: To improve understanding of the natural history of lung cancer with respect to development of cancers and to investigate the patterns of spread and behavior as related to host factors such as immunologic status.

Approach: This program is designed to obtain information with respect to the morphologic, biochemical, and immunologic characteristics related to the development and progression of lung cancer in untreated patients, to elucidate the natural history of the disease in its clinically recognized form, and to develop methods for detecting microfoci of disseminated cancer.

Progress: Prospective studies are being carried out in collaboration with investigators from a variety of disciplines and information is collected which shows indications of increasing our understanding of the tumor biology of lung cancer, of predicting the clinical course on the basis of various laboratory parameters such as immunologic status, and of improving the reliability of detecting disseminated microfoci of tumor cells in patients with apparently localized disease.

Significance for Cancer Research (NCP Objective 5B Approach 4)

These studies are essential from the standpoint of understanding the biology of the disease and the methodology for evaluation of patients so that rational treatment approaches can be investigated on a properly individualized basis.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-972(c), 973,
974, 684, 947, 6050(c)
and 971(c)

1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Radiation Physics Support for Radiation Branch and Other
Laboratories of NCI and NIH

Previous Serial Numbers: Same

Principal Investigator: Dwight W. Glenn, M.S.

Other Investigators: Frederick L. Faw, M.S. and Robert W. Swain, B.S.

Cooperating Units: None

Man Years:

Total:	5
Professional	2

Project Description:

Objectives: To provide radiation physics support for all patient radiotherapy treatments and to provide the tools and techniques of irradiation for other researchers at NIH.

Methods employed: An electronic shop and machine shop are utilized to design and fabricate specialized equipment to determine the necessary irradiation parameters for all irradiators (2, linear accelerators, 1 cobalt unit, 1 neutron generator, 6 x-ray units) and to maintain these irradiators to provide daily service. Two on-line computers and a remote computer terminal provide analytical methods of converting measured parameters to patient treatment application and assist in determining required irradiation modifiers fabricated by the shops. Darkroom facilities are utilized for film dosimetry; other dosimetry methods employ a spectrophotometer, a thermo-luminescent system, activation analysis, calorimetry and the normal ionometric methods.

Service irradiation is performed by the use of a dual x-ray unit; researchers consult on dosimetry and proper methods of irradiating their material.

Proposed Course: A new 6 Mv linear accelerator was installed and the necessary parameters measured to initiate patient treatment. A Van de Graaff generator was removed (100,000 pounds and 5,000 cubic feet) and a cobalt unit relocated. A superficial x-ray unit was removed as well as components for a cesium irradiator (contract cancelled). Service irradiation was performed for 155 researchers at NIH utilizing 4,808 separate exposures (20% increase from preceding year). The high output linear accelerator required much maintenance during the year but the first electron beam treatment was completed for the unit prior to complete shutdown for major overhaul. Three pieces of computer hardware were added to the present system and the software was updated to include patients on new treatment protocols. In the forthcoming year, the high-output linear accelerator will be brought up to the original specifications of 4000 Rads/min at isocenter and emphasis will be placed on electron beam dosimetry and modification of the unit to treat patients at a variety of electron energies. A survey will be conducted to determine the irradiation requirement of researchers at NIH. Peripheral hardware of the computer will be modified and software updated to automate physical parameter input for patient treatment applications.

Honors and awards: U.S. Patent #3678233 awarded on July 18, 1972, for a compensating filter used for "mantle" field irradiation.

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 972(c), 973, 974, 684, 947, 6050(c), and 971(c)
Title: Radiation Physics Support for Radiation Branch and other laboratories of NCI and NIH. (See previous year for specific titles.)

Principal Investigator: D. W. Glenn, F. L. Faw, R. W. Swain

Section: Radiation Physics Section

Lab/Branch: Radiation Branch

Goal: To provide radiation physics support for all patient radiotherapy treatments and to provide the tools and techniques of irradiation for other researchers at NIH.

Approach: An electronic shop and machine shop are utilized to design and fabricate specialized equipment to determine the necessary irradiation parameters for all irradiators (2 linear accelerators, 1 cobalt unit, 1 neutron generator, 6 x-ray units) and to maintain these irradiators to provide daily service. Two on-line computers and a remote computer terminal provide analytical methods of converting measured parameters to patient treatment application and assist in determining required irradiation modifiers fabricated by the shops. Darkroom facilities are utilized for film dosimetry; other dosimetry methods employ a spectrophotometer, a thermoluminescent system, activation analysis, calorimetry, and the normal ionometric methods.

Service irradiation is performed by the use of a dual x-ray unit; researchers consult on dosimetry and proper methods of irradiating their material.

Progress: A new 6 Mv linear accelerator was installed and the necessary parameters measured to initiate patient treatment. A Van de Graaff generator was removed (100,000 pounds and 5,000 cubic feet) and a cobalt unit relocated. A superficial x-ray unit was removed as well as components for a cesium irradiator (contract cancelled). Service irradiation was performed for 155 researchers at NIH utilizing 4,808 separate exposures (20% increase from preceding year). The high output linear accelerator required much maintenance during the year but the first electron beam treatment was completed for the unit prior to complete shutdown for major overhaul. Three pieces of computer hardware were added to the present system and the software was updated to include patients on new treatment protocols.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Treatment modalities and plans were effectuated while striving for optimum utilization.

List of Present Professional Publications:

Serial No. NCI-976(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Treatment of Chronic Lymphocytic Leukemia

Previous Serial Number: None

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1/4
Professional: 1/8

Project Description:

Objectives: The study is directed toward the development of more effective treatment for the management of patients with symptomatic progressive chronic lymphocytic leukemia.

Patient Material: Approximately 100 patients with chronic lymphocytic leukemia have been treated in the Radiation Branch.

Methods Employed: Both standard and investigational approaches have been used with particular emphasis of total body irradiation alone or in combination with chemotherapy.

Major Findings: The information collected on patients treated during the past decade is currently being coded for analysis.

Proposed Course: Continued treatment of patients under study is anticipated with limited accession of new patients into study.

Honors and Awards: None

Publications:

ARTICLES PUBLISHED IN A BOOK

Oldham, R.K., Larson, S.M., Givelber, H.M., and Johnson, R.E.: A

Preliminary Study of ⁵¹-Chromium -Labelled Platelets for the
Evaluation of Splenic Sequestration in Chronic Lymphocytic
Leukemia. J. Nucl Med April, 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-976(c)

Title: The Treatment of Chronic Lymphocytic Leukemia

Principal Investigator: Ralph E. Johnson

Section: Radiation Therapy Service

Lab/Branch: Radiation Branch

Goal: To investigate new approaches for the more effective management of patients with symptomatic, progressive chronic lymphocytic leukemia.

Approach: The utility of various treatment modalities, both alone and in combination, is being examined with the aim of improving both the frequency and completeness of response. The treatment approaches being investigated include total body irradiation, alkylating agents, corticosteroids, and adjuvant splenectomy.

Progress: An increased frequency of hematologic remission has been observed with total body irradiation as compared to other forms of treatment and splenectomy has been demonstrated to have a beneficial effect on tolerance to treatment, even in patients having preoperative platelet survival times within the normal range. Complete hematologic remissions have been obtained in a number of patients and the impact of such responses on the natural history of the disease is under study.

Significance for Cancer Research (NCP Objective 6 Approach 1)

There are no established forms of treatment recognized to alter the course of events and significantly prolong survival for patients with chronic lymphocytic leukemia. The approaches being investigated have at least met the initial requirement for this goal in that complete hematologic remissions have been produced with greater frequency than reported for other types of treatment.

Level of Effort: Professional Man-Years: 1/8

Serial No. NCI-982(c)
1. Radiation Branch
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Computer Storage and Retrieval System for Clinical Data

Previous Serial Number: None

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Sandra K. Johnson, R.N., Frederick Faw, M.S.

Cooperating Units: None

Man Years:

Total: 3/2
Professional: 1/2

Project Description:

Objectives: This project is directed toward the development of a computer system for the storage and retrieval of clinical data on research patients of the Radiation Branch.

Patient Material: Patients entered into protocol studies are having their clinical records reviewed, coded, and stored on tape at the present time. Approximately 200 patients with Hodgkin's disease and 100 patients with malignant lymphoma have computerized records to date.

Methods Employed: Programs for coding and storage of data is being developed by the staff of the Radiation Branch for each of the individual disease entities under study. Prior to final coding, records undergo a minimal dual review by professional staff in an attempt to insure storage of accurate data. The Wilbur program utilizing the DCRT IBM-360 computer is employed and a terminal is located in the Radiation Branch to facilitate continuous editing of data according to changes in patient status and to permit retrieval of information as expeditiously as possible.

Major Findings: Availability of a computer system for data storage and retrieval has and will increasingly play a major role in supporting many of the clinical research activities in the Branch.

Proposed Course: Continued expansion of the program to all ongoing clinical studies and investigation of methods for facilitating the

extrapolation of such a system to general use at NIH and in the medical community.

Honors and Awards: None

Publications: None directly.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-982(c)

Title: Computer Storage and Retrieval System for Clinical Data

Principal Investigator: Ralph E. Johnson, M.D. and Sandra K. Johnson, R.N.

Section: Office of the Chief

Lab/Branch: Radiation Branch

Goal: Development of a computer system for the storage and retrieval of clinical data on research patients treated in the Radiation Branch

Approach: Programs have been written for the coding and storage of information on the epidemiology, natural history, diagnosis, evaluation, treatment, results of therapy, mechanisms of treatment failure, and long-term complications and prognosis of cancer patients entered into the prospective clinical trials ongoing in the Radiation Branch. The DCRT IBM-360 computer is being utilized with a remote terminal being located on the premises of the radiation therapy area, thus permitting continuous editing of the data file in addition to an expeditious means of retrieval.

Progress: The data file is completed for the Hodgkin's disease patient population (nearly 200 patients) and coding has been completed for almost one-half of the 200 patients with non-Hodgkin's lymphomas entered into study to date. It is anticipated that the latter will be completed during the next year in addition to storage of data on the Ewing's sarcoma and chronic lymphocytic leukemia series of patients.

Significance for Cancer Research (NCP Objective 5B Approach 6)

Level of Effort: Professional Man-Years: 1/2

Serial No. NCI-983(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Pathogenesis of Chronic Lymphocytic Leukemia

Previous Serial Number: None

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 1/2

Professional: 1/4

Project Description:

Objectives: This study is directed toward improving the understanding of the epidemiology and natural history of chronic lymphocytic leukemia.

Patient Material: Approximately 100 patients with chronic lymphocytic leukemia have been seen in the Radiation Branch and are available for study.

Methods Employed: Information is currently being collated and coded for computer storage, retrieval, and analysis.

Major Findings: Description of major findings awaits analysis of data once computerization of information has been completed.

Proposed Course: Continued long-term observation of patients entered on prospective treatment protocols is anticipated but no significant number of additional patients will be accessioned into study.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-983(c)

Title: The Pathogenesis of Chronic Lymphocytic Leukemia

Principal Investigator: Ralph E. Johnson, M.D.

Section: Radiation Therapy Service

Lab/Branch: Radiation Branch

Goal: To increase understanding of the epidemiology of chronic lymphocytic leukemia, to study factors associated with the transformation of disease from the indolent to active form, and to document the spectrum of changes
Approach which occur in the host secondary to untreated disease.

Patients with previously untreated chronic lymphocytic leukemia are being studied longitudinally over extended periods of time and serial observations are being made on their general condition, influence of exogenous factors such as infection on the disease process, and the relationship between the immune mechanism and the other parameters of disease activity. The effect of treatment on the host and disease is being evaluated with the attempt to develop more satisfactory means of controlling the direct and indirect consequences of this rather enigmatic clinical problem.

Progress: Information is still being prospectively obtained and no definitive correlations are as yet available. Data on nearly one hundred patients is currently being compiled for analysis with the hopes of at least yielding some of the needed information on the pathogenesis and natural history of the disease.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Level of Effort: Professional Man-Years: 1/4

Serial No. NCI-984(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Epidemiology of the Malignant Lymphomas and Hodgkin's Disease

Previous Serial Number: None

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Sandra K. Johnson, R.N.

Cooperating Units: None

Man Years:

Total: 1/8
Professional: 1/8

Project Description:

Objectives: The purpose of this project is to identify population groups with a high relative risk of developing malignant lymphoma or Hodgkin's disease and factors which may predispose toward these diseases.

Patient Material: Approximately 350 patients entered into clinical trials are available for study.

Methods Employed: An effort is being extended to obtain detailed information, both with respect to the patients' past history and the family history. This information is being computerized to facilitate future analysis.

Major Findings: The in-depth information being collected has made possible heretofore unavailable data on the tonsillectomy history of patients with Hodgkin's disease as correlated with not only the demographic features of the disease but also with the sibling history of tonsillectomy. Other such correlations requiring extensive prospective collection of information should be possible in the future.

Proposed Course: This program will be continued on all patients entered into clinical trial.

Honors and Awards: None

Publications:

ARTICLES PUBLISHED IN A PERIODICAL

Johnson, S.K., and Johnson, R.E.: Tonsillectomy History in Hodgkin's Disease. New Engl. J. Med 287: 1122, 1972 (Nov 30).

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 984(c)

Title: Epidemiology of the Malignant Lymphomas and Hodgkin's Disease

Principal Investigator: Ralph E. Johnson, M.D., Sandra K. Johnson, R.N.

Section: Radiation Therapy Service

Lab/Branch: Radiation Branch

Goal: To identify population groups with a high risk of developing malignant lymphoma or Hodgkin's disease or factors which predispose towards these diseases.

Approach: Information is being prospectively gathered on patients with respect to family history, occupation, infectious disease history, tonsillectomy state, etc., for the purpose of examining factors which may correlate with either the overall incidence of the neoplasm or alternatively certain subsets of patients.

Progress: Data has been collected on approximately 200 patients with Hodgkin's disease and data collection is underway on new cases with non-Hodgkin's lymphomas currently being accessioned into clinical trials. A critical review of the influence of prior tonsillectomy on the development of Hodgkin's disease has recently been completed and reported.

Significance for Cancer Research (NCP Objective^{5A} Approach 1)

The malignant lymphomas constitute one of the more common types of cancer and yet there is virtually no information to date with respect to the epidemiologic aspects. This may relate to the incompleteness of available information and the program here described may detect what must be rather subtle demographic features related to the development of these diseases.

Level of Effort: Professional Man-Years: 1/8

Serial No. NCI-985(c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Diagnostic Assessment of the Malignant Lymphomas and Hodgkin's Disease

Previous Serial Number: None

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Clinical Associates

Cooperating Units: Medicine Branch and Surgery Branch, NCI; Diagnostic Radiology and Nuclear Medicine, Clinical Center, NIH

Man Years:

Total: 4/2

Professional: 2/2

Project Description:

Objectives: This study is directed toward the development of reliable methods for detecting microfoci of cancer and to the assessment of the utility of these diagnostic methods in guiding therapeutic approaches in addition to improving understanding of the natural history of the diseases.

Patient Material: Approximately 350 patients have been entered into diagnostic study.

Methods Employed: A collaborative effort involving the various cooperating units is directed toward each patient and involves a systematic evaluation designed to establish the biologic characteristics of malignant lymphomas and Hodgkin's disease. These methods include radiographic and scanning techniques, biopsy procedures, and other tests as required including exploratory laparotomy on a selective basis.

Major Findings: A comprehensive review is currently underway and some of the findings to date are described in the publications listed below.

Proposed Course: Continued accession of patients with malignant lymphoma into this program is anticipated.

Honors and Awards: None

Publications:

ARTICLES PUBLISHED IN A PERIODICAL

Bagley, C.M., Thomas, L.B., Johnson, R.E., Chretien, P.B., and DeVita, V.T.: Diagnosis of Liver Involvement by Lymphoma: Results in 96 Consecutive Peritoneoscopies. Cancer(In Press)

Milder, M.S., Larson, S.M., Bagley, C.M., DeVita, V.T., Johnson, R.E., and Johnston, G.S.: The Liver-Spleen Scan in Hodgkin's Disease. Cancer(In Press)

Lamoureux, K.B., Jaffe, E.S., Berard, C.W., and Johnson, R.E.: Lack of Identifiable Vascular Invasion in Patients with Extranodal Dissemination of Hodgkin's Disease. Cancer(In Press)

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-985(c)

Title: Diagnostic Assessment of the Malignant Lymphomas and Hodgkin's Disease

Principal Investigator: Ralph E. Johnson, M.D., Clincl Associates

Section: Radiation Therapy Service

Lab/Branch: Radiation Branch

Goal: To develop methods for detecting microfoci of cancer cells which have spread from the primary site.

Approach: Comprehensive diagnostic evaluation using both established and newer investigative techniques is undertaken on all patients prior to initiation of treatment for the purpose of detecting microfoci of involvement. The subsequent treatment is then designed to test the reliability of these methods in locating the microfoci of spread.

Progress: Numerous programs have been completed and are currently underway which are yielding significant information on the patterns of spread of cancer cells from the primary sites as well as the relative merits of the methods themselves. These programs involve collaboration with the Diagnostic Radiology and Nuclear Medicine departments of the Clinical Center, the Medicine Branch and Laboratory of Pathology, NCI, and the Surgery Branch, NCI.

Significance for Cancer Research (NCP Objective 5B Approach 4)

These studies have major implications not only for understanding the biology of cancer, the modes and patterns of spread, and the diagnostic methods which can be utilized for detecting and following the microfoci of spread but also for the selection of properly individualized treatment for each patient.

Level of Effort: Professional Man-Years: 1

Serial No. NCI-6054(c)
1. Radiation Branch
2. Radiation Therapy & Physics
Sections
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Treatment of Non-Hodgkin's Lymphomas

Previous Serial Number: Same

Principal Investigator: Ralph E. Johnson, M.D.

Other Investigators: Frederick L. Faw, M.S. and Dwight W. Glenn, M.S.

Cooperating Units: Medicine Branch, NCI

Man Years:

Total: 6/2

Professional: 4/2

Project Description:

Objectives: To improve the effectiveness of therapy for non-Hodgkin's lymphomas using radiation therapy alone and in combination with chemotherapy.

Patient Material: Approximately 150 patients have been entered into this collaborative study during the past five years.

Methods Employed: Following rigorous diagnostic evaluation, patients are randomly assigned to treatment approaches designed to both evaluate and compare new methods of therapy using radiation therapy or chemotherapy alone or in combination.

Major Findings: New treatment methods employ ultra-wide field techniques including total body irradiation and are demonstrating that radiotherapy has a much more significant role in the management of malignant lymphomas than anticipated. The results of treatment are in part detailed in the publications listed.

Proposed Course: Continued accession of patients and observation of those already entered into study are expected for the next several years.

Honors and Awards: None

Publications:

ARTICLES PUBLISHED IN A PERIODICAL:

Johnson, R.E.: Remission Induction and Remission Duration with Primary Radiotherapy in Advanced Lymphosarcoma. Cancer 29: 31, 1972.

Johnson, R.E.: Radiation Therapy of Generalized Lymphocytic Lymphomas. Amer J Roent 117: 50, 1973.

Johnson, R.E.: Radiation Management of the Lymphomas. Proceedings of Seventh National Cancer Conference. Cancer (In Press)

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-6054(c)

Title: The Treatment of Non-Hodgkin's Lymphomas

Principal Investigator: Ralph E. Johnson, M.D., Frederick L. Faw, M.S.

Section: Radiation Therapy Service and Radiation Physics Section

Lab/Branch: Radiation Branch

Goal: To improve the effectiveness of therapy for the non-Hodgkin's lymphomas, using radiation therapy alone and in combination with chemotherapy.

Approach: The current approach is to examine the comparative usefulness of chemotherapy and radiotherapy when used alone and to identify the mechanisms of treatment failure so that a rational method of combining these modalities can be developed where indicated. This is a collaborative study with the Medicine Branch, NCI.

Progress: Approximately 150 patients have been entered into this collaborative study and entered into prospective protocols involving treatment with radiation therapy and/or chemotherapy. New methods have been developed and tested such as ultra-wide field irradiation techniques and total body irradiation which appear to have considerable utility for selected patients.

Significance for Cancer Research (NCP Objective 6 Approach 1,6)

Level of Effort: Professional Man-Years: 1

Serial No. NCI-6502 (c)
1. Radiation Branch
2. Radiation Therapy Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Study of Ewing's Sarcoma as a Model System in
Experimental Therapeutics for Adjuvant Chemotherapy

Previous Serial Number: Same

Principal Investigators: Ralph E. Johnson, M.D. and Thomas C. Pomeroy, M.D.

Other Investigators: Robert K. Oldham, M.D., and Adelbert Evans, Ph.D.

Cooperating Units: Laboratory of Cell Biology, Division of Cancer Biology
and Diagnosis; and the Armed Forces Radiobiological
Research Institute.

Man Years:

Total: 3/6
Professional: 2/6
Other: 1/6

Project Description:

Objectives: Ewing's sarcoma, though relatively uncommon, has provided a unique "tumor model" for evaluation of adjunctive chemotherapy. The accumulation of a histologically verified group of patients for evaluation of the natural history and behavioral characteristics of the disease can provide information which will be of value in direction of improved methods of therapeutic management of this disease and similar approaches in other forms of malignancy as more efficient chemotherapeutic agents and combination of agents become available.

Patient Material: Sixty-five patients have been admitted to the study with 39 receiving prophylactic chemotherapy, 22 receiving adjunctive chemotherapy for primary and/or metastatic disease. The remaining four patients have been segregated from the Ewing's group with a diagnosis of primitive osteosarcoma.

Methods Employed: In the case of clinically localized disease, intensive systemic chemotherapy has been combined with primary treatment using radiation therapy or surgery. Irradiation of the central nervous system has been evaluated to eradicate microscopic foci of metastatic disease protected from drug effect by the blood-brain barrier.

Laboratory evaluations of participation of the immune system in the

response to treatment are now available and preliminary evidence indicates that microcytotoxicity assays of the patient's lymphocytes against Ewing's and other cell lines, T/B lymphocyte ratios and presence of blocking antibodies will afford prognostic information and aid in treatment decisions. Further, the determination of glycoprotein activity will be exploited to give a measure of total tumor cell volume and activity. Preliminary evidence here shows promise of providing guidance in application of chemotherapy. These findings will be correlated with known prognostic factors of (1) tumor site; (2) age at diagnosis, and where possible, with histology of host reactions in biopsy material; all in relation to clinical response.

Major Findings: Projected five year survival rates of forty percent in the prophylactic therapy group have been maintained with 15 months as the critical period for recurrence. Two patients have relapsed after four years and two years free of clinical disease; these relapses developing concomitantly with onset of menstrual periods in both instances. One previous relapse after fifteen months was after five years free of clinical disease and chemotherapy retreatment following local resection of lung metastasis has resulted in an additional three year clinical disease-free state.

Adriamycin in Phase II evaluation in patients with relapse and unresponsive to standard drugs produced a superlative response with 6/11 obtaining greater than 50 percent tumor regression.

The introduction of Adriamycin in the chemotherapy regime appears to be providing a significant improvement in extending the early disease free period in the prophylactic group and providing disease free periods extending after cessation of all treatment in the metastatic group with 4/10 free of clinical disease from eight to fourteen months following initial treatment. This indicates the possibility of still further improvement in the management of this very malignant tumor by controlling metastatic disease that is clinically evident as well as that present only as disseminated microscopic foci.

Proposed Course: Continuation of patient acquisition for further evaluation of treatment and to provide serial laboratory evaluation of new parameters through the period of therapy and followup. Serial serum samples will be stored for future evaluation of blocking antibodies in the progression to attempts at immunotherapy in a high risk group anticipated determinable by the above described prognostic indicators.

Honors and Awards: None

Publications:

Oldham, R.K., and Pomeroy, T.C.: Treatment of Ewing's Sarcoma With Adriamycin. Cancer Chemo. Rep., Part 1, Vol. 56, No. 5, October 1972.

Johnson, R.E., and Pomeroy, T.C.: Integrated Therapy for Ewing's Sarcoma. Amer. J. Roentgen. 114: 532, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-6502 (c)

Title: The Study of Ewing's Sarcoma as a Model System in Experimental Therapeutics for Adjuvant Chemotherapy

Principal Investigator: Ralph E. Johnson, M.D., and Thomas C. Pomeroy, M.D.

Section: Radiation Therapy Service

Lab/Branch: Radiation Branch

Goal: Determine the role of adjunctive chemotherapy in improving the survival rates in Ewing's sarcoma.

Approach: Patients with primary localized Ewing's sarcoma will be entered on the study for evaluation of adjunctive chemotherapy and prophylactic whole brain irradiation for determination of possibility of control of microscopic metastatic foci of tumor disseminated by the time of diagnosis and in "sanctuary" behind the blood-brain barrier.

A limited number of patients with metastatic disease will also be entered for determination of ability of adjunctive chemotherapy to clear clinically evident metastatic involvement.

In both instances, patients will receive standard radiation therapy to the primary bone involvement, limited radiation to the whole brain, and cyclic chemotherapy with multiple drug combinations.

Progress: Projected five year survival rates of forty percent are being maintained despite occasional relapses after long disease free intervals. Two of three of these have occurred in females concomitant with the onset of menstruation at four and two years after initial therapy.

Phase II studies with adriamycin have provided greater than 50 percent regression in 6/11 patients refractory to other more standard drugs. Recent experience with Adriamycin in the primary treatment program has improved the early disease free percentage in the prophylactic group and has, in addition, produced clinical freedom of disease in the metastatic group with 4/10 patients free of clinical disease ranging from eight to fourteen months after initial treatment.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Level of Effort: Professional Man-Years: 2/6

SUMMARY STATEMENT
ANNUAL REPORT
DERMATOLOGY BRANCH
DCBD, NCI

July 1, 1972 through June 30, 1973

The Dermatology Branch conducts both clinical and basic research on the etiology, diagnosis, and treatment of malignant diseases involving the skin and the host's response to these diseases. The Branch also serves as dermatologic consultant to all other services at the Clinical Center (approximately 800 patients are seen in consultation per year) and to NIH employees through the Employee Health Clinic. The Branch currently includes the Branch Chief, 5 senior investigators, 1 staff fellow and 4 clinical associates.

The Dermatology Branch has made significant contributions toward the objectives of the National Cancer Plan. The major research achievements of the Dermatology Branch for the past year are as follows:

Xeroderma pigmentosum. This rare autosomal recessive dermatologic disease is characterized by pigmentary changes, telangiectasia, atrophy, and tumor formation in response to even minimal sun exposure. Tumors which commonly occur in XP patients (basal and squamous cell carcinomas, malignant melanomas, etc.) are histologically identical with those found in otherwise normal persons. A defect (presumably in an endonuclease) in repair of UV damage to DNA has been found in XP cells. As suggested by the formulators of the National Cancer Plan (Executive Report, Vol. II, Section 1-3. p. II 14), "development of this important area may lead to an understanding of the basic nature of the carcinogenic change...and to the development of effective preventive measures." The NCI Dermatology Branch has the largest series of XP patients on whom complete photobiological, clinical, and laboratory evaluations have been performed. Cells from several tissues have been obtained from these patients, and work in this Branch has demonstrated the suitability of cells other than the customarily used skin fibroblasts for studying the DNA repair defect in XP. Thus, use of peripheral blood lymphocytes rather than dividing cells largely overcomes the problem of the repair synthesis being obscured by semiconservative DNA synthesis. A rapid and sensitive assay for DNA repair synthesis in human lymphocytes was utilized in the Branch and made possible a discovery of significance not only for XP but also for carcinogenesis of any type in which DNA repair is involved. Using this assay, investigators in the Branch were able to demonstrate that, contrary to previous reports, cells from XP patients do not differ from normal in the amount of DNA repair synthesis (as measured by ^3H -thymidine incorporation) but only in the rate of such synthesis. The repair rate in cells from a given XP patient is consistent from one cell type to another; in addition to lymphocytes and skin fibroblasts, studies have been conducted in the Branch on XP epidermal and conjunctival cells. The conjunctiva is a convenient source from which to obtain epithelial cells, since the contaminating stromal cells can be removed with relative ease. Investigators in the Dermatology Branch have discovered a form of XP in which the patient's cells have no demonstrable DNA repair defect, despite the presence

of severe skin disease including numerous malignant tumors. Cells from this patient and other XP patients are being studied in the Branch to determine the role of DNA repair in tumor formation after exposure to ultraviolet radiation of chemical carcinogens which react with and distort DNA.

Genetic complementation studies are being performed by fusing cells from pairs of XP patients. Thus far, 4 complementation groups have been found among the patients whose cells have been studied in the Branch, indicating the existence of at least 4 loci at which mutations can produce the XP repair defect. The 4 complementation groups were found to correlate with the magnitude of the repair defect (as reflected in the rate of DNA repair synthesis) rather than with the clinical types of the diseases (presence or absence of neurological involvement).

Cells from all XP patients studied by the Branch are characterized according to their DNA repair rate, and the patients undergo a thorough clinical evaluation. The cells, together with the clinical classifications, are sent to the American Type Culture Collection for dissemination for use by other investigators.

Malignant Melanoma. Malignant melanoma is a highly malignant tumor in which production of a specific differentiated cell product (melanin) not only continues but is often increased. The melanin granules in tumors are, however, morphologically distinguishable from those found in normal cells. The Dermatology Branch is investigating melanogenesis and its regulation in normal and malignant cells to determine the relationship of this process to melanoma formation; cells from various pigmentary mutants of the mouse have been compared to elucidate the genetic basis for control of melanin production. It has been found that (1) Melanogenesis in normal black mice and the more than 10 pigment mutants studied, followed a common pathway of melanosome formation; the major difference noted among the mutants was the premature termination of melanization of the granules at different stages of melanosome formation; (2) Melanosomes from black mice contain seven proteins, six of which are of extremely high molecular weight. Preliminary results of analysis of melanosomes from amphibians are remarkably similar to those of the mouse, although proteins from (mouse) melanoma melanosomes are quite different from normal - a most interesting observation. We are currently preparing to isolate and characterize melanosomes from normal and malignant human tissue; and (3) Tyrosinase activity in black mice seems to be under the control of a heat-labile, low molecular weight (dialyzable) inhibitor. Tyrosinase activity in albino mice, heretofore assumed to be absent, can be demonstrated when tissue homogenates are pretreated with detergents. This indicates that the c locus (albino) should be regarded (in mice) as a regulator locus rather than a structural locus. In addition, we have determined that melanogenesis in the mouse is dependent upon tyrosinase activity rather than peroxidase activity, as has been suggested by other workers. These studies are currently being expanded to include normal and malignant pigmentary systems in humans, as well as in mice.

T-Cell Leukemias

Another major area of research in the Dermatology Branch is the study of Sezary syndrome and "small cell variant" - chronic lymphocytic leukemias of the T cell type. The Sezary syndrome is a lymphomatous disease characterized by intense generalized reddening and exfoliation of the skin and leukemic cells circulating in the blood. Ultrastructural studies in the Branch have shown the large circulating Sezary cells to have a characteristic convoluted nucleus. The Sezary cell is probably lymphocytic by virtue of its PHA responsiveness in culture, and has been shown to have chromosomes abnormal in both number and structure. Investigators in the Branch employing ultrastructural, cytophotometric, and cytogenetic methods have confirmed and extended these findings. Two types of abnormal circulating cells have been recognized: first, a big type with a convoluted nucleus, about tetraploid DNA values and near tetraploid chromosome counts; second, a "small cell variant" with an indented, irregular nucleus, diploid DNA values and a pseudo or hyperdiploid chromosome count. In addition, these large and small cells appear to have no surface immunoglobulins and thus are not B-lymphocytes in contrast to CLL cells which are predominately B-lymphocytes. An additional patient with "small cell variant" is presently being studied and on the basis of specific surface reactivity with human anti-T antibody has been shown to have a T-lymphocyte disease. Since the T lymphocyte is apparently the important cell in immunological surveillance against neoplasia and the progenitor cell of some specific lymphokines, the recognition of a leukemic patient in which the T cell appears predominate has important implications. In addition, the surface features of these cells and other leukemic cells are being studied with the scanning electron microscope to determine differences from their normal counterparts. In addition, methods have been worked out in the Branch which can define B cells and T cells and histiocytes in tissue. These methods will be important in identifying B cell types in many kinds of pathologic lesions including skin cancers. In the years 1954 through 1969, 144 mycosis fungoides patients were studied and treated in the Branch. Our experiences with these patients have recently been reviewed. The above studies are related to the following Project Areas of the National Cancer Plan: 21C01, 24A06, 34B04, 41A02, 42C03, 56C09, and 66B01.

Immunotherapy of Skin Cancer

Another major area of clinical research within the Branch is the evaluation study of DNCB immunotherapy for basal cell carcinomas. There has been much interest in attempts to induce man's self-defense mechanisms against cancer by producing allergic reactions by a DNCB sensitization. This type of therapy has been reported to cause reversal of some skin tumors. The claim has been made that 90% or more of basal cell carcinomas respond to DNCB therapy and we have attempted to generate data to test this claim. A study underway in our Branch indicates that large nodules of basal cell carcinoma only partially regress, and that papular and superficial basal cell carcinomas respond rather poorly. In fact in 4 of 5 patients studied, only 25% of the basal cell carcinomas responded. Croton oil, a primary skin irritant, was found to provoke about the same number of "cures". These studies are being continued. Concomitant studies on allergic contact dermatitis are closely related and include development of a method for adapting

the DNCB contact sensitization test to the in vitro lymphocyte transformation test. Because DNCB is a primary immunogen and contact sensitizer the combined in vivo - in vitro approach offers unique features for: (1) the study of mediators involved in cellular immunity; (2) the study of allergic contact dermatitis during primary sensitization and anamnestic challenge; (3) the study of tolerance; and (4) approaches similar to those which have proven successful for DNCB may offer in vitro diagnostic tests for other contact allergens and possibly some drug allergies.

In addition, mediators of cellular immunity produced by lymphocytes are being studied as well as the cellular interactions of lymphocytes and macrophages, lymphocytes with lymphocytes, and epithelial cells with leukocytes in relationship to histocompatibility studies which are also being pursued.

Keratohyalin. There are two major morphological constituents unique to the epidermal cell, the tonofilament and the keratohyalin granule. Our research efforts have been directed towards determining the nature of the keratohyalin granule. In marked contrast to studies by previous investigators, we have demonstrated that keratohyalin can be easily extracted and solubilized from epidermis by salt solutions. Further study of the salt solubility properties showed that keratohyalin undergoes in vitro aggregation. Because of this property of undergoing spontaneous aggregation, it is now very easy to isolate purified bovine keratohyalin. Identity of the isolated material as keratohyalin has been confirmed by histochemical, ultrastructural and immunological methods. Physical-chemical studies of isolated keratohyalin reveal that the granule is composed of identical subunits with a molecular weight of 16,900. As suggested from previous histochemical and autoradiographical studies, bovine keratohyalin is rich in serine, arginine, glycine and histidine, and is complexed to RNA. Formation of polymers which range in size up to the 20-mer is apparently a covalent process, while formation of aggregates with molecular weights in the millions of non-covalent.

Present studies are directed at further biochemical characterization, including determining the precise method of cross-linking the polymers. Other studies relating to function of keratohyalin utilize a short-term culture method.

Epidermal Cell Differentiation. Another area of research in the Dermatology Branch has been the investigation of mechanisms governing epidermal cell differentiation. Inhibition of keratinization of embryonic chick skin by vitamin A with subsequent mucus metaplasia is a reproducible model for the study of altered epidermal differentiation. Results to date confirm previous morphologic studies, but also show intra-epidermal mucus glandular development. Metaplasia was inhibited by puromycin and cycloheximide, but not actinomycin D, hydroxyurea, and colchicine, suggesting that control of vitamin A induced metaplasia may be at the translational level. Recent biochemical studies of this process show that DNA synthesis is enhanced within the first 6 hours of exposure, but inhibition or alteration of normal epidermal protein synthesis had not occurred. Analysis of vitamin A effects at later time periods is in progress.

Topical Chemotherapy. (1) Topical BCNU is effective in the treatment of basal cell epithelioma in those patients who developed inflammation during treatment, (2) topical CCNU is effective in psoriasis with production of prolonged remissions, but temporary bone marrow suppression occurred in 2 patients, and (3) topical vitamin A, used occlusively, was irregularly and temporarily effective in psoriasis. In addition, it has been found that patients with Darier's disease exhibit accelerated wound healing and beneficial response to dermabrasion studied.

Scanning and Transmission Electron Microscopy. The structural parameters of cell function and disease are being studied with a wide range of optical methods comprising light microscopy, transmission electron microscopy (TEM), and scanning electron microscopy (SEM). These observations provide: (1) the most detailed descriptive morphological data available with current technology; (2) direct experimental evidence for the physiological roles of certain cells and organelles, and (3) correlative evidence and controls on purity and sampling of cells or cell components being studied by other methods. The development, behavior, fate, and pathology of leukocytes and epithelial cells are our principal concern; these cells play an essential role in normal and diseased skin, and are uniquely suited for experimental study.

A basic study of fine surface morphology of human leukocytes is in progress. Individual normal and leukemic cells are positively identified by familiar Giemsa staining methods and these same cells are then examined by SEM under a variety of conditions, including mitogen stimulation. A new often surprising view of cell form and function is emerging from these studies. SEM studies of the surface features and dermal organization of normal and diseased human epidermis, and of the effects of Vitamin A on cultured chick skin are also in progress, and TEM studies of leukocytes and skin using cytochemistry and quantitative autoradiography are planned.

Several other current SEM projects are designed to develop and test new methods and to broaden and validate interpretation in this unexplored field. These include studies of stimulated thyroid epithelium, Herpes-infected human intestinal organ cultures, and chick embryo cells transformed with Rous sarcoma virus. Collaboration with others in the Dermatology Branch includes the characterization of particulate keratohyalin and cultured epidermal cells, and the development and application of labeled antibody methods.

Serial No. NCI-3613
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Extraction, Isolation and Characterization of Keratohyalin Granules

Previous Serial Number: Same

Principal Investigator: Arthur Ugel, M.D.

Other Investigators: Walter J. Klingler, M.D. and Peter Elias, M.D.

Man Years (July 1, 1972 through June 30, 1973)

Total:	3.2
Professional:	1.2
Other:	2.0

Project Description:

Objectives:

- 1) To develop an in vitro system for short-term culture studies of epidermis.
- 2) To determine conditions and drugs which modify keratinization in vitro using bovine keratohyalin as a biochemical marker.
- 3) To determine the mechanism of polymerization of bovine keratohyalin employing various biochemical methods.
- 4) To evaluate the histochemical, ultrastructural and biochemical properties of human and rat keratohyalin.
- 5) To isolate and characterize human and rat keratohyalin.
- 6) To determine the identity or non-identity of keratohyalin from various animals species by isolation and subsequent biochemical and immunological studies.
- 7) To determine the relationship between bovine keratohyalin and bovine trichohyalin.
- 8) To study the surface structure of bovine keratohyalin using the scanning electron microscope.

Methods Employed:

- 1) Bovine epidermis is prepared as previously described in the presence of Media 199 containing penicillin and streptomycin. Epidermis is minced into approximately 1 mm cubes and then placed in a metabolic shaker. Tritiated precursors are then added to the media. Incorporation is terminated at various time periods, and bovine keratohyalin is isolated by the high-salt in vitro aggregation methods. Specific activities are then determined.
- 2) Short-term culture of epidermis is performed as described above. Modification of incorporation is attempted by adding blocking agents (puromycin, actinomycin), dibutyryl cyclic-AMP, vitamin A acid).
- 3) Bovine keratohyalin is isolated by the high-salt in vitro aggregation method. The isolated keratohyalin polymers are then made water soluble by various chemical manipulation. The water soluble keratohyalin is then studied by further chemical manipulation and subjected to disc gel electrophoresis.
- 4) Human and rat tissue are fixed in paraffin and studied histochemically. Extraction patterns are determined as previously described using serial dilutions of various salt solutions.
- 5) Human and rat epidermis are prepared and extracted in 1.0M potassium phosphate buffer (pH 7.). Aggregates are formed by dialysis against distilled water. The aggregates are solubilized in sodium decyl sulfate and subjected to disc gel electrophoresis.
- 6) Keratohyalin is extracted from various ungulate species. The isolated macroaggregates are studied by biochemical and immunological methods as previously described.
- 7) Sections of cattle epidermis are made, screening for specimens having prominent trichohyalin granules. These sections are then studied by indirect immunofluorescence using antibody specific for bovine keratohyalin.
- 8) Bovine keratohyalin macroaggregates are prepared. Macroaggregates are then examined in the scanning electron microscope.

Major Findings:

- 1) Short term culture of epidermis is possible as demonstrated in our present system which incorporates tritiated histidine into bovine keratohyalin. Preliminaries studies indicate that incorporation is linear. Very short incubation results in labeling of the small molecular weight species, while longer incubations result in the labeling of all species.
- 2) Modification of incorporation is demonstrated by adding puromycin, which completely inhibits the incorporation of tritiated histidine

into bovine keratohyalin. Dibutyryl cyclin-CMP does not significantly alter the incorporation. Vitamin A acid stimulates incorporation at certain levels, and depresses at high levels.

- 3) All attempts to depolymerize the bovine keratohyalin polymers have been unsuccessful. Present studies are directed at specific enzymatic digestions and peptide mapping.
- 4) Human and rat keratohyalin have histochemical properties similar to bovine keratohyalin. Preliminary data indicate similar extraction patterns.
- 5) Extraction of human and rat keratohyalin, and disc gel electrophoresis of the aggregates formed results in the fractionation of numerous proteins (and not a polymeric series as seen in the case of ungulate keratohyalin. Further identification is being studied by the application of histochemical methods to the fractionated proteins.
- 6) Keratohyalin has been isolated from sheep, goat, horse and gnu species. Fractionation of these species results in gel patterns similar to those noted with bovine keratohyalin. All species cross-react immunologically.
- 7) Bovine trichohyalin and bovine keratohyalin cross-react immunologically as determined by indirect immunofluorescence.
- 8) Bovine macroaggregates appear to be oval bodies with a relatively smooth surface.

Significance to Cancer Research:

The epidermis offers a unique opportunity for the study of tumors because of its accessibility and the rapid turnover of epidermal cells. One of the major problems in studying malignancies of epidermis has been the lack of a unique biochemical marker which could be closely monitored. Keratohyalin can be used as such a marker, and, in fact, is absent in basal cell carcinoma, squamous cell carcinoma and psoriasis. With our present methods, we can now evaluate the effects of carcinogens on keratohyalin synthesis. Furthermore, it should be possible to screen various drugs in vitro for possible clinical use in cancer of the skin.

Proposed Course of Project:

Emphasis will be placed on the further biochemical characterization of keratohyalin. Screening of chemotherapeutic drugs will be initiated upon further development of our in vitro system.

Honors and Awards:

Gold Award for exhibit "Isolation and Characterization of Bovine Keratohyalin", 1971, American Academy of Dermatology. A.R. Ugel, M.D., S.B. Guss, M.D., P.M. Elias, M.D., M.A. Lutzner, M.D., W.W. Idler, P.M.

Montague.

Husik Award for paper "Further Characterization of Bovine Keratohyalin", 1972, A.R. Ugel, W.W. Idler.

Publications:

- 1) Ugel, A.R., and Idler, W.W.: Further characterization of bovine keratohyalin. J. Cell Biol. 52: 453-464, 1972.
- 2) Guss, S.B., and Ugel, A.R.: Immunofluorescent antibodies to bovine keratohyalin and immunologic confirmation of homology. J. Histochem. Cytochem. 20: 97-106, 1972.
- 3) Elias, P.M., Montague, P.M., and Ugel, A.R.: In vitro studies on the kinetics, composition, and homology of bovine keratohyalin. Exp. Cell Res. 73: 95-100, 1972.
- 4) Guss, S.B., and Ugel, A.R.: Methanol-fixed paraffin-embedded bovine and human epidermis for antinuclear antibodies. Arch. Derm. 106: 674-677, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3613

Title: Extraction, Isolation, and Characterization of Keratohyalin Granules

Principal Investigator: Arthur R. Ugel, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To characterize keratohyalin, a major component of epidermis, and to apply this basic knowledge to clinical dermatology.

Approach: The epidermis is composed of various layers which are distinguishable at the histochemical level. One of these layers is the stratum granulosum which contains a unique cytoplasmic particle, the keratohyalin granule. This material is very abundant in the epidermis of cattle hooves, and our laboratory has developed methods for its isolation and characterization. By the method of high salt extraction and subsequent in vitro aggregation, it is possible to isolate aggregates which are identical to the in situ keratohyalin granule. Biochemical characterization of the isolated keratohyalin on disc gel electrophoresis indicates that the granule is composed entirely of a set of polymers with a subunit molecular weight of 17,000. The protein has an unusual amino acid composition, and appears to be complexed to a ribonucleotide.

Progress: In addition to further biochemical characterization of keratohyalin, studies are being directed at methods to study the synthesis of keratohyalin in vitro. Synthesis can be modified by various chemicals as has been demonstrated in other organ systems.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Keratohyalin is not seen in basal cell carcinoma or squamous cell carcinoma. Keratohyalin can be used as a biochemical marker, and, in vitro, it should be possible to study drugs as well as carcinogens to determine their effects on keratinization. Such screening may result in a better understanding of the etiology of skin cancer, and may reveal certain drugs which could be used clinically to treat cancer of the skin.

Level of Effort: Professional Man-Years: 1.2

Serial No. NCI-3625
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Epidermal Macromolecular Synthesis in Chick Embryo, and Normal and Diseased (Benign and Malignant) Human Skin: A Biochemical, Autoradiographical Study with Particular Emphasis on the Effects of Vitamin A.

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: Vincent J. Hearing, Jr., Ph.D.

Cooperating Units: None

Man Years (July 1, 1972 through June 30, 1973)

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives:

- 1) To investigate cutaneous macromolecular biosynthesis; and to define the effect of vitamin A in inhibiting keratinization, in embryonic skin, normal adult skin, and in benign and malignant lesions.
- 2) To define certain dermatologic disorders at the biochemical level.

Material:

- 1) 0.2-0.4 mm thick, Castroviejo keratotome slices of normal and diseased human skin. Skin from patients with Darier's Disease (NCI-3643) has been used to date.
- 2) 3-4 mm punch biopsies of normal and diseased human skin (NCI-3643).
- 3) Metatarsal shank skin from 13-21 day chick embryos.

Methods Employed:

Chick embryo skin or skin from patients with Darier's Disease was incubated both with and without Vitamin A in a water bath shaker at 37°C for

2-6 hours in the presence of labelled precursors. Occasional experiments involved 3 day organ culture of chick skin with and without vitamin A prior to exposure to radioactivity.

After incubation, specimens were prepared for biochemical extraction and analysis. Various macromolecular fractions (DNA, RNA, protein, and glycoprotein) were isolated using standard methodology. The specific activities of these fractions were determined, and separation and characterization of individual molecular species was defined using polyacrylamide gel electrophoresis. Quantitative and qualitative changes in response to experimental design could be readily followed by banding patterns assayed both spectrophotometrically and by liquid scintillation counting.

Major Findings:

Preliminary electrophoretic analyses have indicated that several major protein components found in normal skin are lacking in involved skin of Darier's. This data, when considered with the overall hypometabolism found in Darier's patients, might indicate that certain metabolic processes are inhibited in these patients. In response to vitamin A treatment, although the overall rate of protein synthesis was only slightly increased in Darier's skin, whatever metabolic block was functioning in the suppression of the several missing proteins, was effectively removed, and substantial synthesis of these proteins began in these tissues. It has been known for some time that occasional patients with Darier's Disease respond to topical and systemic therapy with vitamin A. This study may provide the first clue as to the mechanism of action of vitamin A in the treatment of Darier's Disease. The identity of these critical proteins remains to be established. We are currently studying the synthesis of the various RNA's in these tissues, and the response of their synthesis to vitamin A treatment, in an effort to determine at what level these inhibitory factors and the derepression mechanisms are located. These studies, it is hoped will indicate the metabolic abnormality in Darier's Disease, and hopefully, an effective way to correct it.

We have studied the effects of vitamin A on embryonic chick skin at the biochemical level. At the microscopic level, treatment with vitamin A causes mucous metaplasia, involving the inhibition of normal keratinization and the simultaneous stimulation of mucin production. Electrophoretic analysis indicates that synthesis of several major polypeptides is turned off by vitamin A administration (this would account for the inhibition of keratinization very nicely), but to date, we have been unable to find the stimulation of any protein which might represent the prosthetic group of mucin (a glycoprotein). On the other hand, when H³-fucose (one of the sugars in epithelial mucins) is used as precursor, it can be seen, again with the aid of gel electrophoresis, that several glycoproteins are decreased in synthesis with vitamin A administration, while one low molecular weight glycoprotein (which is absent in normal tissue) is synthesized very actively. While further work is needed, these results would seem to indicate that vitamin A causes mucus metaplasia primarily by inhibiting keratinization, and at the same time

allowing mucin formation to occur, presumably by the glycosylation of pre-existing protein in the chick epidermal cell.

Our results indicate that vitamin A acts at the level of cellular control mechanisms, and, at least in the case of embryonic chick skin, normal and Darier's human skin, causes basic redifferentiation of the cell at the molecular level.

Significance to Cancer Research:

Since carcinogenesis is an instance of altered differentiation, studies of vitamin A-induced redifferentiation, both in human and chick skin, may serve as an excellent model for investigations of cellular control mechanisms which relate to carcinogenesis.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Biology of cells capable of forming cancers - steps preceding cancer development at cell transformation (and other) body sites: determine rates of (cancer) cell transformation and percent of rates leading to tumor formation.

Proposed Course of Project:

In chick skin, continued biochemical analysis will be done in order to more accurately characterize the epidermal mucin formed, and the mechanism by which vitamin A inhibits normal epidermal protein synthesis and stimulates mucin biosynthesis. The effect of vitamin A on epidermal cell kinetics may be studied.

In Darier's Disease, more patients will be studied to confirm our preliminary observations, and to characterize the major epidermal proteins that are lacking in diseased skin. Skin from normal volunteers may be needed for comparative analysis.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3625

Title: Epidermal Macromolecular Synthesis in Chick Embryo, and Normal and Diseased (Benign and Malignant) Human Skin: A Biochemical, Autoradiographical Study with Particular Emphasis on the Effects of Vitamin A.

Principal Investigator: Gary L. Peck, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To biochemically define the mechanism of action of vitamin A in inhibiting keratinization in embryonic skin, normal skin, and in benign and malignant lesions of skin.

Approach: Chick embryo skin or skin from patients with Darier's disease was incubated both with and without vitamin A in a water bath shaker at 37°C for 2-6 hours in the presence of labelled precursors. Occasional experiments involved 3 day organ culture of chick skin with and without vitamin A prior to exposure to radioactivity. After incubation, specimens were prepared for biochemical extraction and analysis. Various macromolecular fractions (DNA, RNA, protein, and glycoprotein) were isolated using standard methodology. The specific activities of these fractions were determined, and separation and characterization of individual molecular species was defined using polyacrylamide gel electrophoresis. Quantitative and qualitative changes in response to experimental design could be readily followed by banding patterns assayed both spectrophotometrically and by liquid scintillation counting.

Progress: By polyacrylamide gel electrophoresis, it was found that several major protein components of normal skin are lacking in Darier's disease. Exposure to vitamin A stimulates the production of these components in the diseased tissue in vitro. Vitamin A inhibits normal epidermal protein synthesis in chick skin. Furthermore, incubation with ³H-Fucose, a precursor sugar of epithelial mucins, revealed that one peak of low molecular weight glycoprotein is synthesized very actively in response to vitamin A.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Vitamin A is known to inhibit carcinogenesis. The mechanism of action of vitamin A in inhibiting keratinization may be identical to its antineoplastic activity.

Level of Effort: Professional Man-Years: 0.5

Serial No. NCI-3630
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Vitamin A Acid Induced Mucous Metaplasia of Embryonic Chick Metatarsal Skin in Organ Culture: A Histochemical and Scanning and Transmission Electron Microscopic Study

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: Bruce K. Wetzell, Ph.D., Peter M. Elias, M.D.

Cooperating Units: None

Man Years (July 1, 1972 through June 30, 1973)

Total: 1.0

Professional: 0.5

Other: 0.5

Project Description:

Objectives:

- 1) To investigate the mechanisms governing epidermal cell differentiation.
- 2) To morphologically investigate the mechanism by which vitamin A alters epidermal cell differentiation with resultant inhibition of keratinization.
- 3) To determine if epidermal cells are capable of both mucin and keratin production, or whether the two processes are mutually exclusive.
- 4) To study the early changes of vitamin A induced mucous metaplasia at the ultrastructural level.

Material:

Metatarsal shank skin (devoid of feathers) is obtained from 13-14 day chick embryo.

Methods Employed:

Chick metatarsal skin is cultured in vitro and stimulated to undergo mucous

metaplasia by vitamin A acid at varying doses. Specimens are taken at varying time intervals and evaluated for mucin production by the use of special stains, such as PAS (with and without diastase), Alcian Blue, Toluidine Blue, and Colloidal iron. Specimens are also fixed in glutaraldehyde for subsequent examinations under the transmission and scanning electron microscopes.

Major Findings:

- 1) Using 30 international units of vitamin A acid per milliliter of culture media, it was found that mucous metaplasia occurred with 2 days in in vitro incubation. Maximum metaplasia took place by 6 days.

As little as 1 I.U. produced metaplasia in 6 days. Metaplasia was inhibited or reduced greatly at room temperature, or in the absence of serum. We have studied specimens of chick skin harvested prior to 2 days of incubation and treated with low dosage vitamin A in order to determine the earliest mechanism involved in mucus metaplasia. Ultra-structurally, we found no specific mechanism operative, other than increased amounts of rough endoplasmic reticulum and polyribosomes indicating increased protein synthetic activity.
- 2) Microscopically, the control specimen keratinized normally with the formation of thick bundles of tonofilaments, keratohyalin granules, and a multi-layered stratum corneum. Vitamin A acid treated specimens did not undergo keratinization. There was no tendency of the columnar basal cells to flatten as they approached the surface. Neither stratum corneum, nor keratohyalin granules were formed. Tonofilaments were present in small, fine bundles. In addition, there was increased folding of the nuclear membrane, and increased amounts of rough endoplasmic reticulum and polyribosomes. Mucin was present in the cytoplasm as membrane-bound electron dense material.
- 3) In addition to mucin formation there seems to be a glandular metaplasia in the epidermis with formation of acini. This morphologic change has not been demonstrated previously. Our standard system now utilizes 13-14 day old chick embryos treated with 20 i.u. vitamin A acid in Eagle's Minimal Essential Media with 5% fetal calf serum. Cultures are harvested at 2-3 days. 16 day and older chick embryo skin manifested a selective basal cell layer response to vitamin A.
- 4) Vitamin A induced mucus glandular metaplasia was inhibited by the addition of puromycin or cycloheximide to the culture medium; actinomycin D, hydroxyurea, and colchicine failed to inhibit this process. Thus, protein synthesis is necessary for metaplasia, while mitosis, and DNA and RNA synthesis are not.
- 5) Scanning electron microscopy revealed that metaplastic changes were visible as early as one day after vitamin A exposure. Individual epidermal cells lost attachments to their neighbors, became spherical, and developed surface villi. Selective basal cell layer effect was observed

in 17 day chick embryos treated with vitamin A. Although the superficial stratified squamous layer of the epidermis was unresponsive to vitamin A, it was thrown into large folds by the hyperplastic masses of underlying metaplastic cells.

Significance to Cancer Research:

- 1) Vitamin A is known to inhibit carcinogenesis and to be of value in the treatment of malignancy. The mechanisms of action of vitamin A in inhibiting keratinization may be related to its antineoplastic activity.
- 2) The Objective and Approach of the National Cancer Plan that this project most closely supports is 3:1 Biology of cells capable of forming cancers - steps preceding cancer development at cell transformation (and other) body sites: determine rates of (cancer) cell transformation and percent of rakes leading to tumor formation.

Proposed Course of Project:

Continued ultrastructural examination of the above material.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3630

Title: Vitamin A Acid Induced Mucous Metaplasia of Embryonic Chick Metatarsal Skin in Organ Culture: A Histochemical and Scanning and Transmission Electron Microscopic Study.

Principal Investigator: Gary L. Peck, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To morphologically investigate the mechanism by which vitamin A alters epidermal cell differentiation with resultant inhibition of keratinization.

Approach: Chick metatarsal skin is cultured in vitro and stimulated to undergo mucous metaplasia by vitamin A acid at varying doses. Specimens are taken at varying time intervals and evaluated for mucin production by the use of special stains, such as PAS (with and without diastase), Alcian Blue, Toluidine Blue, and Colloidal iron. Specimens are also fixed in glutaraldehyde for subsequent examinations under the transmission and scanning electron microscopes.

Progress: Microscopically, the control specimen keratinized normally with the formation of thick bundles of tonofilaments, keratohyalin granules, and a multi-layered stratum corneum. Vitamin A acid treated specimens did not undergo keratinization. There was no tendency of the columnar basal cells to flatten as they approached the surface. Neither stratum corneum, nor keratohyalin granules were formed. Tonofilaments were present in small, fine bundles. In addition, there was increased folding of the nuclear membrane, and increased amounts of rough endoplasmic reticulum and polyribosomes. Mucin was present in the cytoplasm as membrane-bound electron dense material. Scanning electron microscopy revealed that metaplastic changes were visible as early as one day after vitamin A exposure. Selective basal cell layer effect was observed in 17 day chick embryos treated with vitamin A.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Vitamin A is known to inhibit carcinogenesis. The mechanism of action of vitamin A in inhibiting keratinization may be identical to its antineoplastic activity.

Level of Effort: Professional Man-Years: 0.5

Serial No. NCI-3631
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Treatment of Skin Cancer with Topical BCNU, and CCNU

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1972 through June 30, 1973)

Total: .01
Professional: .01
Other: 0

Project Description:

Objectives:

To evaluate the therapeutic effectiveness and toxicity of BCNU and CCNU administered topically to the skin surface of the above patients.

Agents:

0.1%, 0.2% and 0.4% Bis-Chloro-ethyl-nitrosourea; and 0.1%, 0.4% and 1.0% chloroethyl-nitrosourea; in 30% liquid petrolatum, 70% white petrolatum.

Material:

- 1) Basal Cell Carcinoma
- 2) Nevroid Basal Cell Carcinoma Syndrome
- 3) Xeroderma pigmentosum with cutaneous carcinoma

Methods Employed:

- 1) Patients must have pathologic confirmation of the clinical diagnosis.
- 2) They must have normal hematologic, hepatic, and renal function.
- 3) Lesions on eyelids and canthi are not included.

- 4) Patients are cautioned to avoid sun.
- 5) Ointments are applied twice daily to affected areas for 8 weeks.
- 6) Laboratory tests are done before, during, and 6 weeks after therapy.
- 7) Serial photographs are taken.

Major Findings:

- 1) A total of 12 patients were included in this study. Nine received only BCNU ointment. One received only CCNU ointment; the remaining two received CCNU ointment initially and were given BCNU later as a second course of therapy. Five of the 12 patients were treatment failures by other modalities, including surgery and topical 5-FU in propylene glycol. Four of the patients had had X-ray therapy of their acne earlier in life. Four other patients had moderate to severe actinic skin. Two patients had the basal cell nevus syndrome; one had xeroderma pigmentosum. Most patients had lesions of the exposed areas of face and neck. Many patients also had lesions on the trunk and extremities. None of the 12 had the standard variety of BCE histologically; the remaining three were superficial BCE, morphea type of BCE, and the fibroepithelioma type of BCE (Pinkus). There were nine males, three females, varying in age from 23-65.
- 2) Results of treatment are as follows:
BCNU 0.1% 26 lesions 10 cures 15 partial responses one no response
CCNU 1.0% 7 lesions 1 cure 5 partial responses one no response
Partial response is defined as visible flattening of the tumor mass.
The ten BCNU cures were in four patients.
- 3) To date, neither recurrences, nor signs of chronic toxicity have been observed.
- 4) Severe contact dermatitis has occurred in some patients with 0.1% BCNU ointment. The dermatitis appeared from day 2 to day 33 in the different patients. Several patients have not developed any cutaneous irritation. In three patients tumors disappeared without antecedent skin inflammation. Usually the patients who had no skin reaction to the medication had little to no therapeutic response. This supports the clinical impression derived from experience with topical 5-FU: No inflammation, No response.
- 5) The main difficulty in conducting this study was the lack of an assay system for the stability of the ointment. The active drugs are unstable in water or propylene glycol, therefore, petrolatum is the only suitable vehicle, and it is not possible to extract the active drug from the petrolatum ointment. This made establishing of the shelf-life difficult. By experience we determined that ointments over one month were not stable.

Significance to Cancer Research:

- 1) This is an exploratory study of a new drug in skin cancer prompted by a desire to find a superior agent to 5-Fluorouracil in propylene glycol which is in widespread topical use.
- 2) We would also hope to contribute to the knowledge of the clinical pharmacology of this agent. Skin cancer should prove to be a valuable lesion for the screening of this and future topical chemotherapeutic agents because the tumors are observable, measurable, and therefore, can be used as an objective indicator of response to therapy. Furthermore, the patients can be expected to be in good health, which would allow for long term studies.
- 3) The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone) and in combinations in the cure and palliation of cancer.

Proposed Course of Project:

Concluded except for continued observation of test subjects to determine recurrence rate and for any signs of chronic toxicity.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3631

Title: Treatment of Skin Cancer with Topical BCNU, and CCNU

Principal Investigator: Gary L. Peck, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To evaluate the therapeutic effectiveness and toxicity of BCNU and CCNU administered topically to the skin surface of the above patients.

Approach: A total of 12 patients were included in this study. Nine received only BCNU ointment. One received only CCNU ointment; the remaining two received CCNU ointment initially and were given BCNU later as a second course of therapy. Five of the 12 patients were treatment failures by other modalities, including surgery and topical 5-FU in propylene glycol. Four of the patients had had X-ray therapy of their acne earlier in life. Four other patients had moderate to severe actinic skin. Two patients had the basal cell nevus syndrome; one had xeroderma pigmentosum. Most patients had lesions of the exposed areas of face and neck. Many patients also had lesions on the trunk and extremities. None of the 12 had the standard variety of BCE histologically; the remaining three were superficial BCE, morphea type of BCE, and the fibroepithelioma type of BCE (Pinkus). There were nine males, three females, varying in age from 23-65.

Progress: Results of treatment are as follows:

BCNU 0.1% 26 lesions 10 cures 15 partial responses one no response

CCNU 1.0% 7 lesions 1 cure 5 partial responses one no response

Partial response is defined as visible flattening of the tumor mass. The ten BCNU cures were in four patients. To date, neither recurrences, nor signs of chronic toxicity have been observed.

Significance for Cancer Research (NCP Objective 6 Approach 1)

This is an exploratory study of a new drug in skin cancer prompted by a desire to find a superior agent to topical 5-Fluorouracil, which is in widespread use.

Level of Effort: Professional Man-Years: .01

Serial No. NCI-3632
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Toxic Epidermal Necrolysis and Graft-Versus-Host Reaction

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: P.M. Elias, M.D., G.P. Herzig, M.D., R.G. Graw, Jr., M.D.

Cooperating Units: Hematology Supportive Care Branch, NCI

Man Years (July 1, 1972 through June 30, 1973)

Total:	.05
Professional:	.05
Other:	0

Project Description:

Objectives:

To study Graft-Versus-Host Reaction (GVH) and Toxic Epidermal Necrolysis (TEN), and to investigate the relationship between them.

Methods Employed:

- 1) Taking of history, and repeated physical examinations of a patient with acute lymphocytic leukemia, treated with allogeneic bone marrow transplantation, who developed GVH, and subsequently, TEN.
- 2) Review of literature
- 3) Examination with the electron microscope of multiple skin biopsies taken at various time intervals and at various skin sites.

Major Findings:

- 1) This is the first documented case in which the usual morbilliform eruption associated with sheet-like desquamation of the epidermis, typical of TEN.
- 2) Early in the clinical course, cutaneous histopathology was typical for GVH.

- 3) Later in the clinical course, biopsies taken from intensely erythematous sites, near areas of massive epidermal desquamation showed more extensive epidermal destruction, consistent with the diagnosis of TEN.
- 4) Support for our thesis linking TEN in this patient with his GVH, comes from animal studies in which experimental GVH in hamsters and monkeys developed TEN. Furthermore, a case of GVH was found in the literature in which a bullous eruption, in retrospect compatible with TEN, developed.
- 5) Electron microscopic findings: In biopsies taken at distances greater than 10 cm. from the blister edge, ultrastructural changes were limited to the basal cell layer. The entire basal layer was involved in a uniform manner, while the spinous, granular, and horny layers appear virtually uninvolved. Mitochondrial swelling coarsening of tonofibrils, and increased prominence of ribosomes was noted in all basal cells. The nuclei appeared contracted, with more convoluted nuclear membranes; increased density of chromatin was noted. Biopsies closer to the blister edge displayed considerable intercellular edema, and occasional early bulla formation. Lymphocytes were occasionally seen within the basal layer, but only rarely above. Increased numbers of Odland bodies and abnormal keratohyalin granules were noted in the superficial epidermis. In summary, the initial pathologic events are in the basal layer, and it appears that the mononuclear infiltrate may play a significant pathogenetic role.

Significance to Cancer Research:

- 1) Immunosuppressed patients, including treated cancer patients, who are transfused or transplanted with immunocompetent cells, may expect to develop GVH. In view of this case report, we may now expect some of them to develop TEN as well.
- 2) The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy surgery and radiation (alone) and in combinations in the cure and palliation of cancer.

Proposed Course of Project:

Electron microscopic examination is continuing. Specimens from patients with either TEN or GVH are also being studied.

Honors and Awards:

First Prize - 1971 North American Clinical Dermatological Society Memorial Essay Contest.

Publications:

- 1) Peck, G.L., Herzig, G.P., and Elias, P.M.: Toxic Epidermal Necrolysis in a Patient with Graft-Versus-Host Reaction. Arch. Derm. 105: 561-569, 1972

- 2) Peck, G.L., Elias, P.M., Graw, Jr., R.G.: Graft-Versus-Host reaction and Toxic Epidermal Necrolysis. *Lancet* II (7782): 1151-1153, 1972
- 3) Elias, P.M., Arndt, K.A., Peck, G.L.: Scalded Skin Syndrome in Adults. *New Eng. J. Med.* 288: 582-583, 1973

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3632

Title: Toxic Epidermal Necrolysis and Graft-Versus-Host Reaction

Principal Investigator: Gary L. Peck, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To study Graft-Versus-Host Reaction (GVH) and Toxic Epidermal Necrolysis (TEN), and to investigate the relationship between them.

Approach: Clinical and ultrastructural study of patients with Graft-Versus-Host Reaction (GVH) and/or Toxic Epidermal Necrolysis (TEN).

Progress: A case of GVH leading to TEN was observed. Support from our thesis linking TEN in this patient with his GVH, comes from animal studies in which experimental GVH in hamsters and monkeys developed TEN. Furthermore, a case of GVH was found in the literature in which a bullous eruption, in retrospect compatible with TEN, developed. Electron microscopic findings revealed that the initial pathologic events are in the basal layer, and it appears that the mononuclear infiltrate may play a significant pathogenetic role.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Immunosuppressed patients, including treated cancer patients, who are transfused or transplanted with immunocompetent cells, may expect to develop GVH. In view of this case report, we may now expect some of them to develop TEN as well.

Level of Effort: Professional Man-Years: .05

Serial No. NCI-3633
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Treatment of Psoriasis with Topical CCNU. (IND-7003)

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: Stephen Guss, M.D. and Douglas Key, M.D.

Cooperating Units: None

Man Years (July 1, 1972 through June 30, 1973)

Total:	.01
Professional:	.01
Other:	0

Project Description:

Objectives:

To evaluate safety and effectiveness of topical CCNU in psoriatic patients.

Agents:

0.4% and 1.0% CCNU in 30% liquid petrolatum and 70% white petrolatum.

Material:

Adult patients with psoriasis. Females must be past the childbearing age.

Methods Employed:

- 1) Patients must have psoriasis previously unresponsive to standard therapy.
- 2) They must have normal kidney, liver, and bone marrow function.
- 3) Medication will be applied twice daily until clear.
- 4) Patients will avoid sun exposure to prevent phototoxicity, if any.

- 5) Laboratory tests measuring bone marrow, liver, and kidney function will be obtained before, during, and 6 weeks after cessation of therapy.
- 6) Serial photographs will be obtained.

Major Findings:

- 1) Seven of 9 patients treated for longer than 2 weeks cleared completely. The 2 patients who did not clear had severe psoriasis of the palms and soles. Four additional patients were treated for less than 2 weeks.
- 2) Severe irritation of the skin surrounding the psoriatic plaques occurred. This was especially marked in the intertriginous areas.
- 3) Subsequent to the cutaneous irritation, persistent pigmentation, telangiectasia, and cutaneous pain developed as well as temporary bone marrow suppression.
- 4) Four patients have remained free of disease in CCNU treated areas since October, 1970 to the present.
- 5) No signs of delayed or chronic toxicity have appeared.

Significance to Cancer Research:

- 1) This study is part of an ongoing search for an effective topical therapy for psoriasis.
- 2) It is hoped that this study will contribute to the pharmacology of chemotherapeutic agents.
- 3) The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone) and in combinations in the cure and palliation of cancer.

Proposed Course of Project:

Concluded except for continued observation of test subjects - for any chronic toxicity and for determination of length of remission.

Honors and Awards: None

Publications:

Peck, G.L., Guss, S.B. and Key, D.J. Topical Lomustine in the Treatment of Psoriasis. Arch. Derm. 106: 172-176, 1972

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3633

Title: Treatment of Psoriasis with Topical CCNU

Principal Investigator: Gary L. Peck, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To evaluate safety and effectiveness of topical CCNU in psoriatic patients.

Approach: Patients with psoriasis were treated with topical 1% lomustine (CCNU) in petrolatum in a double blind study against placebo.

Progress: Seven of 9 patients treated for longer than 2 weeks cleared completely. The 2 patients who did not clear had severe psoriasis of the palms and soles. Four additional patients were treated for less than 2 weeks. Severe irritation of the skin surrounding the psoriatic plaques occurred. This was especially marked in the intertrigenous areas. Subsequent to the cutaneous irritation, persistent pigmentation, telangiectasia, and cutaneous pain developed as well as temporary bone marrow suppression. Four patients have remained free of disease in CCNU treated areas since October, 1970 to the present. No signs of delayed or chronic toxicity have appeared.

Significance for Cancer Research (NCP Objective 6 Approach 1)

This study is part of an ongoing search for an effective topical therapy for psoriasis. It is hoped that this study will contribute to the pharmacology of chemotherapeutic agents.

Level of Effort: Professional Man-Years: 0.01

Serial No. NCI-3637
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ultrastructure of Melanogenesis in Normal and Abnormal
Mammalian Tissues

Previous Serial Number: Same

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Other Investigators: Marvin A. Lutzner, M.D.

Cooperating Units: None

Man Years (July 1, 1972 through June 30, 1973)

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives:

To examine melanogenesis by electron microscopy in various pigment forming tissues both in normal and abnormal conditions. Further elucidation of the process of melanin granule formation in mammals should be achieved by careful examination of various melanogenic systems.

Methods Employed:

Tissues selected for examination are dissected out and fixed immediately in glutaraldehyde, then osmium, followed by dehydration, embedding, sectioning and staining by conventional procedures. Histochemical staining of tissues is also carried out by published EM techniques.

Major Findings:

A series of pigment mutants of the mouse has been investigated which are analogues of normal and abnormal human pigmentation: black (normal), white (albino) and beige (Chediak-Higashi syndrome), among others. The results of this phase of our work indicate that a common pathway of melanogenesis exists which is common to all pigment mutants thus far studied. A fibrillar premelanosome is formed, upon which melanin is uniformly deposited. Eventually the melanized fibers become so thick

that fusion between them takes place, and all structural detail is masked. In the mutants studied, the basic difference from normal melanogenesis as described above, is the premature termination of melanin synthesis at one of several points. This results in incompletely formed, rather than structurally aberrant, granules. In the beige animal, many normally forming granules fuse together, resulting in giant granules. Since the fusion of other types of granules in these animals is noted, the mechanism of fusion is not restricted to pigment granules alone. The current theory of the formation of these giant granules is a membrane abnormality which induces the fusion of normal granules.

Significance to Cancer Research:

The objective and approach of the National Cancer Plan that this project most closely supports is 3.1: Study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning. Characterization of normal mammalian melanogenesis is essential before any work on abnormal melanogenesis can be properly evaluated and interpreted. The long range aim of this project is the study of melanomas and other types of pigmentary disorders in humans. Since both melanotic and amelanotic melanomas apparently involve structurally atypical melanin granule formation, we feel that our current research may be informative regarding the normal and abnormal metabolic pathways involved in melanogenesis in these tissues, and ultimately, provide clues for the metabolic control of these cancers.

Proposed Course of Project:

The work on the ultrastructural aspects of melanogenesis of the pigment mutants of the mouse is virtually completed. Future work planned involves a similarly approached study on human melanogenesis, both in normal and cancerous conditions.

Honors and Awards: None

Publications:

1. Lutzner, M.A.: Ultrastructure of the development of the normal black and giant beige melanin granules in the mouse. In Riley, V. (Ed.): Pigmentation. New York, New York, Appleton-Century Crofts, 1970.
2. Lutzner, M.A.: Ultrastructure of giant melanin granules in beige mutants of the mouse. In Montagna, W. (Ed.): VIII Pigment Cell Conference, New York, New York, Appleton-Century Crofts, 1972.
3. Hearing, V.J., Phillips, P. and Lutzner, M.A.: The fine structure of melanogenesis in coat color mutants of the mouse. J. Ultra. Res., 1973. (In press).

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3637

Title: Ultrastructure of Melanogenesis in Normal and Abnormal Mammalian Tissues.

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To examine melanogenesis by electron microscopy in various pigment forming tissues both in normal and abnormal conditions. Further elucidation of the process of melanin granule formation in mammals should be achieved.

Approach : Tissues selected for examination are dissected out and fixed immediately in glutaraldehyde, then osmium, followed by dehydration, embedding, sectioning and staining by conventional procedures. Histochemical staining of tissues is also carried out by published techniques. Pigment mutants studied thus far were bred in our laboratories from appropriate stocks obtained from commercial suppliers.

Progress: The project, which has been completed on the murine melanogenesis, is currently being geared to study in a similar manner the process of human melanogenesis, both in normal and cancerous conditions.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Characterization of normal mammalian melanogenesis is essential before any work on abnormal melanogenesis can be properly evaluated and interpreted. The long range aim of this project is the study of melanomas and other types of pigmentary disorders in humans. Since both melanotic and amelanotic melanomas apparently involve structurally atypical melanosome formation, we feel that this research may be informative regarding the normal and abnormal metabolic pathways involved in melanogenesis in these tissues.

Level of Effort: Professional Man-Years:

0.0

Serial No. NCI-3638
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of DNA repair in normal human cells and in cells from patients with xeroderma pigmentosum

Previous Serial Number: Same

Principal Investigator: Jay H. Robbins, M.D.

Other Investigators: Kenneth H. Kraemer, M.D.

Cooperating Units: Hayden G. Coon, Ph.D., Laboratory of Cell Biology, DNCB, NCI; Rosemary Cuddy, Program Analysis and Evaluation Branch, DCG, NCI

Man Years (July 1, 1972 through June 30, 1973)

Total:	2.7
Professional:	1.7
Other:	1.0

Project Description:

Objectives:

To study the DNA excisional repair process in normal cells and in cells from patients with xeroderma pigmentosum (XP). The role of this process in repair of damage caused by ultraviolet (UV) radiation, alkylating agents and other chemicals is being evaluated. The relationship between abnormalities in the function of this process and the development of skin and other tumors in humans is being investigated.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Nature and modification of precancerous state and determination of mechanisms accounting for high degree of stability of cell functioning.

Materials:

Cells are obtained from hospitalized patients and outpatients. Cells studied include: XP and normal peripheral blood lymphocytes, dermal fibroblasts, epidermal cells, basal cell carcinoma cells and malignant melanoma cells. Lymphocytes from patients with chronic lymphocytic leukemia are also studied.

Methods Employed:

'Purified' lymphocytes are separated from other blood leukocytes by passage through nylon fiber columns. Epidermal cells are obtained from dermatome shavings and are studied as an intact tissue. All other cells used are cultured by standard tissue culture methods. Fibroblast heterokaryons are formed by virus-induced fusion of cells from pairs of XP patients. The cells are cultured and exposed to UV light or to alkylating agents or other chemicals and then incubated with tritiated thymidine. DNA repair is assayed by measuring the UV-induced unscheduled thymidine incorporation by autoradiography and scintillation spectroscopy.

Major Findings:

We have evaluated the clinical manifestations and the DNA repair rates of 6 more XP patients, thereby bringing the number of XP patients studied by us at the NIH to 14 and the number of XP kindreds to 11. New clinical types of XP have been discovered among these patients. Our series of XP patients is the largest in which a valid attempt can be made to relate the various clinical manifestations of the disease to the patients' repair rates. Skin fibroblasts from all of these patients (and from related family members) are sent to the American Type Culture Collection for dissemination to other investigators.

Our studies have shown that patients within the same family have similar repair rates and similar clinical manifestations. Unrelated XP patients, however, usually have different degrees of skin and other organ system involvement and different repair rates. There does not appear to be any obvious correlation between the clinical manifestations of XP and the degree of impairment of the DNA repair process. However, 12 of the 14 patients have defective DNA repair while two have no demonstrable defect in repair as measured by UV-induced unscheduled thymidine incorporation. The latter two XP patients may have a defect in such incorporation which is too small for us to detect. Alternatively, they may have a defect in some other aspect of DNA repair, or they may have a defect which does not involve any DNA repair process.

Methods have been developed to study DNA repair in cells other than peripheral blood lymphocytes and dermal fibroblasts. Thus, conjunctival, melanoma, and basal cell carcinoma cells have been cultured in vitro, and their UV-induced thymidine incorporation has been determined. Dermatome shavings of intact epidermis have been used successfully to evaluate DNA repair in vitro in XP epidermal cells without disrupting the architecture of that organ. The relative rate of UV-induced thymidine incorporation into any one tissue of an individual is similar to that into any other tissue from that individual.

Genetic complementation studies are being performed in vitro in heterokaryons formed by fusing fibroblasts from pairs of XP patients. Thus, by combining two different XP cells it is possible to ascertain whether they have sufficiently different genotypes to permit restoration of the DNA

repair process in the nuclei of the multinucleated heterokaryons. Two cell strains whose nuclei fail to show such restoration of the DNA repair process are said to be in the same complementation group and can be expected to have a genetic lesion affecting the identical gene product. Thus far, four complementation groups have been found among the patients whose cells we have studied, indicating the existence of at least four loci at which mutations can produce the XP repair defect. The four complementation groups were found to correlate with the magnitude of the repair defect (as reflected in the rate of UV-induced thymidine incorporation) rather than with the clinical types of the disease (e.g. presence or absence of neurological involvement). The recognition and identification of these complementation groups should be of considerable value in the biochemical investigations which are currently underway and which are designed to recognize and purify the defective repair enzyme(s) in XP.

Studies have been conducted on the rate of DNA repair in leukemic lymphocytes from patients with chronic lymphocytic leukemia. Our findings to date indicate that these cells have a normal rate of such repair in contrast to reports in the literature which claim these cells have markedly increased rates of repair.

Studies have also been conducted to determine the optimal conditions for culturing lymphocytes. These studies have shown that one of the most frequently used culture media, tissue culture medium 199, is markedly inferior to several other media in supporting blastogenesis and DNA synthesis in response to phytohemagglutinin, allogeneic cells, and commonly-employed antigens. In addition, a microculture assay technique has been developed so that DNA synthesis can be measured in leukocyte cultures containing one-tenth to one-twentieth the usual number of cells. This microculture system is of particular value when either the leukocytes or blastogenic factors are available only in limited quantities.

Significance to Bio-medical Research and the Program of the Institute:

UV-radiation is believed to be the major cause of the most common type of human cancer, cancer of the skin. Patients with XP are particularly susceptible to the carcinogenic action of UV-radiation and develop multiple malignancies on sun-exposed areas of skin. Normal human cells have an excisional repair process which rapidly repairs DNA damage, while most XP patients have a marked impairment in the rate of such repair. This process is involved not only in repair of UV-induced DNA damage but also in repair of damage by certain chemical carcinogens. Understanding the relationship between DNA repair deficiency and skin tumor development in XP patients would, therefore, elucidate the role of DNA repair in preventing in other humans those cancers which may be due to certain chemical and physical carcinogens.

Proposed Course of Project:

Continuation of research as indicated in the foregoing.

Honors and Awards: None

Publications:

- 1) Robbins, J.H., Levis, W.R. and Miller, A.E. Xeroderma pigmentosum epidermal cells with normal UV-induced thymidine incorporation. J. Invest. Dermatol. 59: 402-408, 1972
- 2) Robbins, J.H. and Levis, W.R. Inherent inability of chronic lymphocytic leukaemia lymphocytes to respond to phytohaemagglutinin. Intnl. Arch. Allergy 43: 845-858, 1972
- 3) Robbins, J.H. Measurement of DNA synthesis in leucocyte microcultures. J. Clin. Path. 25: 1075-1078, 1972
- 4) Robbins, J.H. and Burk, P.G. Relationship of DNA repair to carcinogenesis in xeroderma pigmentosum. Cancer Research 33: 929-935, 1973
- 5) Robbins, J.H., Elias, P.M. and Ornstein, L. Intercellular lacunae: sequestered microenvironments in stimulated leukocyte cultures. Experientia 29: 212-213, 1973

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3638

Title: Studies of DNA Repair in Normal Human Cells and in Cells from Patients with Xeroderma Pigmentosum.

Principal Investigator: Jay H. Robbins, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To study DNA repair in normal cells and in cells from patients with xeroderma pigmentosum; to elucidate the relationship between DNA repair and carcinogenesis.

Approach: Xeroderma pigmentosum (XP) is a rare autosomal disease in which the patients develop numerous malignancies on sun-exposed areas. Most patients with XP are unable to repair ultraviolet (UV)-induced damage to their DNA at a normal rate. We study the ability of XP patients' cells to repair UV-damaged DNA in vitro by measuring the rate of incorporation of radio-active thymidine into their DNA during the repair process by autoradiography and liquid scintillation spectroscopy. The XP patients are studied clinically with particular emphasis on evaluating the dermatologic, neurologic, ophthalmologic and endocrinologic manifestations of their disease. Attempts are made to correlate the patients' clinical findings with their DNA repair rate. In addition, genetic complementation studies with cultured fibroblasts are conducted to determine whether the patients have similar or dissimilar genetic mutations which result in deficient DNA repair.

Progress: Our NIH patient series, which now comprises 14 patients and 11 kindreds, is the largest group of XP patients in which complete clinical and photobiological studies have been conducted and correlated. New clinical types of XP and DNA repair deficits have been discovered in patients of this series. Methods have been successfully employed to study DNA repair in vitro in numerous tissues other than just the commonly employed dermal fibroblasts, e.g., epidermal, conjunctival, peripheral blood, malignant melanoma and basal cell carcinoma cells. Genetic complementation studies using multinucleate, fused XP fibroblasts have indicated the existence of at least four loci at which mutations can produce the XP repair defect.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Normal human cells have an excisional repair process which rapidly repairs certain types of DNA damage caused by UV-radiation and by certain chemical carcinogens, while most XP patients have a marked impairment in the rate of such repair. Understanding the relationship between DNA repair deficiency and skin tumor development in XP patients would, therefore, elucidate the role of DNA repair in preventing in other humans those cancers which may be due to certain chemical and physical carcinogens.

Level of Effort: Professional Man-Years: 1.7

Serial No. NCI-3641
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunotherapy of Skin Cancers with DNCB

Previous Serial Number: Same

Principal Investigator: William D. Terry, M.D.

Other Investigators: William R. Levis, M.D., Gary L. Peck, M.D., Kenneth H. Kraemer, M.D., Walter G. Klingler, M.D.

Cooperating Units: Immunology Branch, NCI

Man Years (July 1, 1972 through June 30, 1973)

Total:	0.8
Professional:	0.8
Other:	0

Project Description:

Objectives:

- 1) To determine the efficacy of 2, 4 dinitrochlorobenzene (DNCB) on cutaneous malignancies and premalignant growths in subjects sensitized to DNCB.
- 2) To evaluate DNCB as a useful model for the study of immunotherapy of certain human cancers and possibly lead to additional immunotherapy studies.
- 3) The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone) and in combinations in the cure and palliation of cancer.

Material:

- 1) Patients with multiple skin cancers
- 2) 2, 4 Dinitrochlorobenzene
- 3) acetone
- 4) acid mantle creme
- 5) croton oil

Methods Employed:

1. Sensitization: Subjects with multiple skin cancers are sensitized by challenge with DNCB (2,000 ug in 0.1 ml of acetone or 50 mgms/day of acid mantle creme).
2. Titration of Dose for Application: Serial dilutions of DNCB beginning with 100 ug in 0.1 ml acetone or 50 mgm of the 1:10,000 DNCB in aqueous base are applied as patch tests to 1 cm areas of normal skin 10 to 14 days following sensitization.
3. Methods of Challenge to tumors: The initial concentrations of DNCB applied to the tumor surface is 10 fold lower than the lowest concentration which elicits a positive patch test as determined by step 2. The DNCB is applied to the tumor either in acetone or in acid mantle creme. Application will be twice daily for a period of up to eight weeks. Residual lesions will be followed for 4 more weeks. If they do not regress completely, another course of therapy will be undertaken.
4. In all individuals with multiple skin lesions a number of lesions will serve as controls. These lesions will be treated either with acetone alone or with acid mantle creme which does not contain DNCB. Some lesions will not be treated at all and some lesions will be treated only with a primary irritant such as croton oil or Na lauryl sulfate.

Major Findings:

113 tumors in 5 dinitrochlorobenzene (DNCB) sensitized subjects with multiple discrete basal cell carcinomas were treated daily for 8 week periods with topical DNCB. 36 of the 113, or 32%, showed complete clinical regression, with follow up periods of 5 to 18 months to date. An additional 29% (33/113) showed partial regression. A sixth subject with uncountable confluent basal cell carcinomas responded well to DNCB, but accurate quantification on an individual tumor basis was not possible: DNCB induced only 15-25% complete regression in 4 of the 5 accurately quantitated test subjects, whereas 21 of 30, or 70%, of basal cell carcinomas challenged with DNCB underwent complete regression in a fifth patient. In addition to variation of response between patients, there was variation of response of even adjacent tumors on the same patient.

Controls in this study included 62 untreated tumors, both adjacent and distant from treated tumors, which showed no evidence of regression. Eleven tumors treated with inert vehicles did not respond. One subject with over 100 nodular basal cell carcinomas became tolerant to DNCB and failed to respond to high concentrations of DNCB. Finally, croton oil a primary irritant that induces inflammation without evidence of cell mediated immunity, did cause complete regression in 6 of 26 (23%) of treated tumors.

The mechanism of DNCB-induced regression of basal cell carcinoma remains unknown. The finding that croton oil, a primary irritant, also induces

tumor regression suggests that part of the therapeutic benefit derived from DNCB may be the result of non-specific inflammation, independent of the cell-mediated immune quality of allergic contact dermatitis. The lack of effect in a tolerant subject suggests that DNCB is by itself not chemotherapeutic or cytostatic. The disappearance of locally invasive human cancer following the therapeutic imposition of a controlled allergic contact dermatitis provides a useful model for the study of immunoneoplastic regression in man.

Significance to Bio-Medical Research and the Program of the Institute:

Contact sensitizers such as DNCB produce a delayed type hypersensitivity reaction. Delayed or cellular immunity appears to be an important type of host immunity against tumor in man as well as other mammals. The mechanism whereby DNCB induces regression of basal cell carcinomas is unknown but may be related to the delayed type hypersensitivity provoked around, on and in the challenged tumor. The use of DNCB and possible other contact sensitizers offers a tool to control cellular immunity around cutaneous malignancies and to ultimately eradicate these tumors.

Proposed Course of Project:

In the future, it might be possible to extend the principles learned from immunotherapy of cutaneous malignancies to a variety of other less accessible malignancies. However, the immediate course of this project will be to continue using DNCB on more patients, varying the concentration and scheduling of the DNCB and control solutions, and ultimately attempting other contact sensitizers such as Pentadecacatechol. Because of the finding that some tumors disappear after challenge with croton oil, a primary irritant, studies with croton oil and other primary irritants will be extended.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3641

Title: Immunotherapy of Skin Cancers with DNCB

Principal Investigator: William D. Terry, M.D., and William R. Levis, M.D.
Section:

Lab/Branch: Immunology Branch and Dermatology Branch

Goal: To evaluate DNCB as a useful model for the study of immunotherapy of certain human cancers and possibly lead to additional immunotherapy studies.

Approach :

(1) Sensitization: Subjects with multiple skin cancers are sensitized by challenge with DNCB (2,000 ug in 0.1 ml of acetone or 50 mgms/day of acid mantle creme).

(2) Methods of Challenge to tumors: The initial concentrations of DNCB applied to the tumor surface is 10 fold lower than the lowest concentration which elicits a positive patch test. The DNCB is applied to the tumor either in acetone or in acid mantle creme. Application will be twice daily for a period of up to eight weeks. Residual lesions will be followed for 4 more weeks. If they do not regress completely, another course of therapy will be undertaken.

Progress: Thirty-six of 113 basal cell carcinomas, or 32% showed complete clinical regression, with follow up periods of 5 to 18 months to date. An additional 29% (33/113) showed partial regression. DNCB induced only 15-25% complete regression in 4 of the 5 accurately quantitated test subjects, whereas 21 of 30, or 70%, of basal cell carcinomas challenged with DNCB underwent complete regression in a fifth patient. In addition to variation of response between patients, there was variation of response of even adjacent tumors on the same patient. One subject with over 100 nodular basal cell carcinomas became tolerant to DNCB and failed to respond to high concentrations of DNCB. Finally, croton oil, a primary irritant that induces inflammation without evidence of cell mediated immunity, did cause complete regression in 6 of 26 (23%) of treated tumors.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Contact sensitizers such as DNCB produce a delayed type hypersensitivity reaction. Delayed or cellular immunity appears to be an important type of host immunity against tumor in man as well as other mammals. The mechanism whereby DNCB induces regression of basal cell carcinomas is unknown but may be related to the delayed type hypersensitivity provoked around, on and in the challenged tumor. The use of DNCB and possible other contact sensitizers offers a tool to control cellular immunity around cutaneous malignancies and to ultimately eradicate these tumors.

Level of Effort: Professional Man-Years: 0.8

Serial No. NCI-3642
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Treatment of Psoriasis with Topical Vitamin A Acid

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: Douglas J. Key, M.D. and Stephen B. Guss, M.D.

Cooperating Units: None

Man Years (July 1, 1972 through June 30, 1973)

Total:	.01
Professional:	.01
Other:	0

Project Description:

Objectives:

To evaluate safety and effectiveness of topical vitamin A acid in psoriatic patients.

Agents:

0.05% to 0.3% vitamin A acid (Eastman) in Unibase (Parke-Davis)

Material:

Adult patients with psoriasis. Females must be past the childbearing age.

Methods Employed:

- 1) Patients must have psoriasis previously unresponsive to standard therapy.
- 2) They must have normal kidney, liver, and bone marrow function.
- 3) Medication will be applied twice daily until clear.
- 4) Patients will avoid sun exposure to prevent phototoxicity, if any.
- 5) Laboratory tests measuring bone marrow, liver, and kidney function

will be obtained before, during, and 6 weeks after cessation of therapy.

- 6) Serial photographs will be obtained.

Major Findings:

- 1) No preferential beneficial response was seen in the treatment of 14 psoriatic patients using vitamin A acid cream without plastic occlusion either (1) at a concentration of 0.05% for 12 weeks in a double blind manner against unibase control, or (2) at concentrations varying from 0.1 to 0.3% for 6 weeks at any given dosage level in comparison with a corticosteroid cream.
- 2) Three of nine patients treated with 0.25% vitamin A acid cream under plastic occlusion in a double blind manner showed definite evidence of clearing limited to vitamin A acid treated areas. This response, which was just evident 2 to 4 weeks after onset of therapy, was short-lived in two of these patients, who also failed to respond to subsequent courses of therapy.

Significance to Cancer Research:

- 1) This study is part of an ongoing search for an effective topical therapy for psoriasis.
- 2) It is hoped that this study will contribute to the pharmacology of chemotherapeutic agents.
- 3) The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.1: Develop and utilize methodologies of immunology, chemotherapy, surgery and radiation (alone) and in combination in the cure and palliation of cancer.

Proposed Course of Project:

Concluded

Honors and Awards: None

Publications:

Peck, G.L., Key, D.J., Guss, S.B.: Topical Vitamin A Acid in the Treatment of Psoriasis. Arch. Derm. 107: 245-248, 1973

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3642

Title: Treatment of Psoriasis with Topical Vitamin A Acid.

Principal Investigator: Gary L. Peck, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To evaluate safety and effectiveness of topical vitamin A acid in psoriatic patients.

Approach: Patients with psoriasis were treated with various concentrations of Vitamin A ointment either in a double-blind manner against placebo or in a corticosteroid cream comparison study.

Progress: No preferential beneficial response was seen in the treatment of 14 psoriatic patients using vitamin A acid cream without plastic occlusion either (1) at a concentration of 0.05% for 12 weeks in a double blind manner against unibase control, or (2) at concentrations varying from 0.1 to 0.3% for 6 weeks at any given dosage level in comparison with a corticosteroid cream. Three of 9 patients treated with 0.25% vitamin A acid cream under plastic occlusion in a double blind manner showed definite evidence of clearing limited to vitamin A acid treated areas. This response, which was just evident 2 to 4 weeks after onset of therapy, was short-lived in two of these patients, who also failed to respond to subsequent courses of therapy.

Significance for Cancer Research (NCP Objective 6 Approach 1)

This study is part of an ongoing search for an effective topical therapy for psoriasis. It is hoped that this study will contribute to the pharmacology of chemotherapeutic agents.

Level of Effort: Professional Man-Years: .01

Serial No. NCI-3643
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Wound Healing, Dermatome Stripping, and Dermabrasion Therapy
in Darier's Disease (Keratosis Follicularis)

Previous Serial Number: Same

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: L. Dellon, M.D., P. Chretien, M.D., I. Cohen, M.D.,
W.G. Klingler, M.D., K. Kraemer, M.D., R. Edelson, M.D.,
P. Elias, M.D.

Cooperating Units: Surgery Branch, NCI

Man Years (July 1, 1972 through June 30, 1973)

Total: .15
Professional: .15
Other: 0

Project Description:

Objectives:

- 1) To observe the response of Darier's Disease to surgical therapy
- 2) To study the effect of experimental wounding on the clinical course of both involved and uninvolved skin.
- 3) To determine if the accelerated healing of dermatome stripped diseased skin in Darier's disease is due to an accelerated epidermal migration.

Material:

Normal and diseased skin in a patient with Darier's Disease

Methods Employed:

- 1) Dermatome Stripping in patient 1. Strips of skin were taken from a 62-year-old female patient with Darier's disease using a Castroviejo ophthalmologic keratome (Storz Instrument Co.). Strips were approximately 2 cm. in width, 5 cm. in length and 0.2 mm. thick, determined by using a 0.2 mm. spacer in the shaving head. Sites included several

areas of the back, upper abdomen and hips. Bleeding was controlled with pressure only. Telfa gauze dressings were used for one to two days, and no other topical therapy was applied. The wounds were then left open, measured and photographed daily.

Incisional Wound Healing. Multiple linear incisions, approximately one cm. in length, were made in diseased skin areas of the right anterior forearm and in clinically uninvolved skin of the left anterior forearm. Because the involved skin surface is more irregular than uninvolved skin, and because the epidermis is variably thickened in dermatoses such as Darier's disease, the standard blade guard device can not be used to ensure equal depth of incisions. We, therefore, chose minimal capillary bleeding as a measure of comparability of incision depth. Telfa gauze was the only dressing employed. Four mm. punch biopsies were taken at zero time, and 3, 6, 12, 18, 24, 48 and 72 hours after wounding. Each biopsy was divided transversely to the wound into two approximately equal pieces. One piece was fixed in cold phosphate-buffered glutaraldehyde, post-fixed in osmium tetroxide, imbedded in Epon 812, and 1-2 u thick sections were stained with Azure II-methylene blue. The other half was routinely fixed in formaldehyde and imbedded in paraffin prior to sectioning and staining. Sections were cut coronally to the incision.

- 2) **Dermabrasion Therapy** in patient 2. A 26 year old man with hypertrophic cornifying Darier's disease especially severe on arms and legs was treated with surgical dermabrasion under general anesthesia several times. Rate of wound healing was measured by serial 6 mm punch biopsies of circular defects produced by 3 mm punches, and by serial punch biopsies taken from dermatome stripped sites of diseased and uninvolved skin punches.
- 3) **Dermatome Stripping** in patient 3. A 37 year old woman with severe, confluent, inflammatory, vesicular Darier's disease was treated with dermatome stripping of her legs under general anesthesia. Rate of wound healing was measured by the methods used in patient 2.

Major Findings:

- 1) **Dermatome Stripping** in patient 1. Involved sites healed more rapidly than uninvolved skin following dermatome stripping. Two weeks after stripping, the uninvolved site remained larger and more erythematous than the involved site. Furthermore, a zone approximately 10 cm. wide of previously diseased skin around stripped sites on the left hip was observed to have clinically regressed by this time. Clinical regression of involved skin surrounding dermatome stripped sites was repeatedly observed.

Five months after the initial stripping experiment, the patient had a generalized exacerbation of her disease, which spared previously dermatomed sites of uninvolved skin. Clinically, the uninvolved stripped sites are now hypopigmented and slightly atrophic. Histologically, the epidermis and pilosebaceous apparatus appear normal except for diminution of the rete ridge pattern and flattening of the papillary dermis.

Incisional Wound Healing. Superficial linear incisions healed more quickly in diseased sites (12 hours) than in uninvolved sites (48 hours) indicating accelerated epidermal migration.

- 2) Diseased sites healed more rapidly than uninvolved sites, using both methods of measuring rate of wound healing. Incisional wound healing studies were not done because of the difficulty in locating incisions in diseased tissue after 1-2 days. 3 mm defects in diseased skin healed in 2 d, and in uninvolved skin in 3 d. The patient responded well to dermabrasion and has less than 10% recurrence after 11 months of observation. Peripheral clearing, as seen in patient 1, was not observed.
- 3) Dermatome stripping was well tolerated producing long lasting (up to 6 months) disease free remissions. Peripheral clearing has occurred in this patient following dermatome stripping. Wound healing was more rapid in the diseased skin, but somewhat slower than patient 2.

Significance to Cancer Research:

- 1) Knowledge of the controls governing cell migration after injury are essential to the understanding of malignant growth.
- 2) The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Biology of cells capable of forming cancers - steps preceding cancer development at cell transformation (and other) body sites: determine rates of (cancer) cell transformation and percent of rakes leading to tumor formation.

Proposed Course of Project:

- 1) Ultrastructural examination of the above material.
- 2) Confirmation of these findings in other patients with Darier's disease.

Honors and Awards: None

Publications:

Peck, G.L., M.D. and Elias, P.M., M.D.: Wound Healing in a Patient with Darier's Disease: Preliminary Observations. In Maibach, H. I. and Rovee, D. (Eds.): Epidermal Wound Healing. Chicago, Ill., Year Book Medical Publishers, 1972, pp. 357-363.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3643

Title: Wound Healing, Dermatome Stripping, and Dermabrasion Therapy in Darier's Disease (Keratosis Follicularis)

Principal Investigator: Gary L. Peck, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To observe the response of Darier's disease to experimental wounding and to surgical therapy.

Approach: Patients with severe, recalcitrant, confluent Darier's disease are treated with either dermabrasion or dermatome stripping. Rate of wound healing is measured by histologic examination of sequential punch biopsies taken after (1) incisional wounds, (2) wounds made by 3 mm dermal punches, or (3) sites of dermatome stripped skin.

Progress: Prolonged remissions have been obtained with both forms of surgical therapy. Wound healing is accelerated in Darier's disease. Two of 3 patients have shown peripheral clearing of their disease after dermatome stripping. Dermatome stripped sites of previously uninvolved skin remained disease free in one patient, although surrounding skin became severely involved with an acute flare of Darier's disease.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Knowledge of the controls governing cell migration after injury are essential to the understanding of malignant growth.

Level of Effort: Professional Man-Years: 0.15

Serial No. NCI-3644
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Establishment of a Screening System for Topical Chemotherapeutic Agents in the Therapy of Skin Cancer, Mycosis Fungoides, and Psoriasis

Previous Serial Number: None

Principal Investigator: Gary L. Peck, M.D.

Other Investigators: Eugene J. Van Scott, M.D., Howard I. Maibach, M.D., Phillip Frost, M.D., Gerald Weinstein, M.D., Irwin Freedberg, M.D., Howard Baden, M.D. and Henry Roenigk, M.D.

Cooperating Units: Temple University, University of California Medical Center (San Francisco), University of Miami - Mt. Sinai Hospital, University of Miami, Boston Beth Israel Hospital, Massachusetts General Hospital and Cleveland Clinic

Man Years (July 1, 1972 through June 30, 1973)

Total: .10
Professional: .10
Other: 0

Project Description:

Objectives:

The establishment of a scientific program in which present and future chemotherapeutic agents may be screened topically for antimitotic potential, toxicity, and possible therapeutic use initially in malignant skin diseases, such as cutaneous carcinoma and mycosis fungoides, and subsequently in benign hyperplastic diseases such as psoriasis.

Patients Needed:

At least 10 adult men or women for each chemotherapeutic agent tested topically in the treatment of skin cancer, mycosis fungoides or psoriasis. Females must be past the childbearing age.

Methods Employed:

After chemotherapeutic agents have been supplied by the Chemotherapy Branch, NCI, and after these agents have been screened for biological activity and safety in experimental models, then large scale clinical testing will be done in mycosis fungoides, psoriasis, and skin cancer. It is anticipated that the initial steps in this project will be patch test screening for biological activity in patients with psoriasis and mycosis fungoides and percutaneous absorption studies with radio-labelled chemotherapeutic agents in laboratory animals.

The clinical protocols of this large scale clinical testing have not been formulated as yet. However, the basic design will be similar to NCI-3631 and NCI-3633.

Major Findings:

In progress

Significance to Cancer Research:

- 1) This program is a national cooperative study designed to discover superior topical chemotherapeutic agents than those which are currently available in the treatment of skin cancer, mycosis fungoides, and psoriasis.
- 2) We would also hope to contribute to the knowledge of the clinical pharmacology of these agents. Skin cancer should prove to be a valuable lesion for the screening of this and future topical chemotherapeutic agents because the tumors are observable, measurable, and therefore, can be used as an objective indicator of response to therapy. Furthermore, the patients can be expected to be in good health, which allow for long term studies.
- 3) The Objective and Approach of the National Cancer Plan that this project most closely supports is 6.2: Determine new sources of chemical agents new means of their selection and additional screens for their selection.

Proposed Course of Project:

The entire design of this screening system, including preclinical screens and large scale clinical testing, is currently being established with the assistance of the associate investigators and a committee of pharmacologists, toxicologists, and chemotherapists. Merger of this program with a similar one in the Arthritis Institute is anticipated.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3644

Title: Establishment of a Screening System for Topical Chemotherapeutic Agents in the Therapy of Skin Cancer, Mycosis Fungoides, and Psoriasis.

Principal Investigator: Gary L. Peck, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: The establishment of a program to topically screen chemotherapeutic agents for possible therapeutic use in cutaneous carcinoma, mycosis fungoides, and subsequently in benign hyperplastic diseases such as psoriasis.

Approach :

After chemotherapeutic agents have been supplied by the Chemotherapy Branch, NCI, and after these agents have been screened for biological activity and safety in experimental models, then large scale clinical testing will be done in mycosis fungoides, psoriasis, and skin cancer. It is anticipated that the initial steps in this project will be patch test screening for biological activity in patients with psoriasis and mycosis fungoides and percutaneous absorption studies with radiolabelled chemotherapeutic agents in laboratory animals.

Progress: Screening system not yet established.

Significance for Cancer Research (NCP Objective 6 Approach 2)

This program is a national cooperative study designed to discover superior topical chemotherapeutic agents than those which are currently available in the treatment of skin cancer, mycosis fungoides, and psoriasis. We would also hope to contribute to the knowledge of the clinical pharmacology of these agents.

Level of Effort: Professional Man-Years: 0.1

Serial No. NCI-3647
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The fine surface morphology, ultrastructure, and cytochemistry of normal and diseased leukocytes during development, in the circulation, and in sites of leukocytic infiltrates.

Previous Serial Number: Same

Principal Investigator: Bruce Wetzell, Ph.D.

Other Investigators: William R. Levis, M.D.

Cooperating Units: None

Man Years (July 1, 1972 through June 30, 1973)

Total:	2.5
Professional:	0.5
Other:	2.0

Project Description:

Objectives:

To define the surface ultrastructure of leukocytes under a wide range of conditions and thereby provide fresh insight into the physiology of normal and leukemic cells; also, to characterize definitively the origin, contents, and fate of the characteristic granules of neutrophils, eosinophils, and basophils as an index of the development, function and fate of these cells.

Methods Employed:

Buffy coat cells from the peripheral blood of normal humans were diluted in media with autologous serum, allowed to settle on coverslips at 38°C for periods of two minutes to two days, and fixed *in situ* by the addition of an equal volume of 6% aldehyde. The washed coverslips were stained with Giemsa, photographed as wet mounts, then critically dried and prepared for SEM. In this way, individual cells previously identified by light microscopy were examined by SEM. In some cases phytohemagglutinin was included in the medium. SEM studies have been initiated on leukocytes from bone marrow, exudates, macerated lymphoid tissues, tissue culture, and patients with leukemia. Also, representative yields of fixed leukocyte suspensions were deposited and washed on nucleopore filters,

trapped in a thin film of water, quick-frozen, and lyophilized for SEM. Some additional specimens of normal and leukemic leukocytes from bone marrow, peripheral blood, and tissue culture have been prepared for transmission electron microscopy (TEM) with a variety of fixatives.

Major Findings:

Normal human peripheral leukocytes adherent to a coverslip after incubation for 1 hour have been studied intensively. The wide variation in shape and fine surface detail seen among leukocytes of each series is remarkable, and many forms of leukocytes from different series resemble each other closely. These observations were largely unexpected, due to poor sampling and shallow plane projection images of previous TEM and light microscopy (LM). Positive identification of the cells by independent means (such as LM mapping) was shown to be absolutely essential for SEM studies. Unequivocal identification of cells in scanning electron micrographs as members of each leukocyte series, lymphocytes undergoing blastogenesis, dying cells, platelets, megakaryocytes, and myeloid and erythroid precursors has been accomplished by means of our mapping procedures. The forms of these cells clearly reflect adherence, pinocytosis, and cell interaction and provide a wealth of new information. For example, when mixed leukocyte suspensions are fixed only two minutes after they are placed on the coverslip the few adherent cells are mostly monocytes and most of these have flattened drastically onto the coverslip with a smooth upper surface. By five minutes many flattened monocytes have extended long (up to 50 μ m) thin processes along the coverslip with distinctive distal flattened expansions. This peculiar form is less common after 15 minutes of settling and is only rarely encountered after 1 hour. Thus, marked asymmetry can develop almost immediately from rounded cells in suspension, and the widely published views of a gradual change (hours to days) from rounded monocyte to flattened macrophage must be reconsidered.

Cells fixed in acrolein appeared significantly more turgid and erect than those in parallel specimens fixed conventionally in glutaraldehyde. The somewhat shrivelled appearance of the latter suggests shrinkage (the tonicity of the fixatives was essentially the same) although this judgement must be verified by quantitative data. Subjectively, acrolein-fixed leukocytes more closely resemble the living cells.

When supernatants from coverslip preparations of mixed leukocyte suspensions are examined by SEM on nucleopore filters, a distinctive population of small smooth cells poorly represented on the coverslips is seen. These cells would correspond to the small "T" lymphocytes known to adhere poorly to glass substrates.

Initial experiments designed to monitor fixation procedures by light microscopy in thin perfusion chambers have confirmed the feasibility of this approach and have shown no gross artifact introduced by our fixation procedures.

Significance to Cancer Research:

SEM study of buffy coat cells in culture offers a novel and informative view of these cells undergoing attachment, motion, interaction, phagocytosis, response to physical and chemical change, blastogenesis, and degeneration, and provides a new basis for comparison with leukemic cells. The key roles of lymphocytes and macrophages in immune responses to malignant growth and therapy are largely dependent on cell surface phenomena and cell interaction -- both uniquely amenable to study by SEM and ancillary methods.

The characteristic granules of neutrophils, eosinophils and basophils are the principal indices of differentiation and the principal effector organelles of these cells; a thorough understanding of the origin and nature of these granules is necessary to define and interpret the differentiation of normal granulocytes, the cytopathology of chronic granulocytic leukemia and related disorders, and to help clarify the nature and functions of granulocytic infiltrates in skin and other tissues in malignant diseases.

Proposed Course of Project:

Our success in developing methods for the SEM study of positively identified leukocytes, and our initial experience with those from the peripheral blood of normal individuals will be applied in studies of normal development in the hematopoietic tissues, exudates and culture systems, and especially in a survey of leukemic conditions. A major study of leukocyte granulogenesis is planned employing quantitative autoradiography, cytochemistry and TEM. Light microscopic monitoring of preparative conditions and the dynamics of leukocyte adherence, motility, interaction and growth are planned with the use of thin perfusion chambers.

Honors and Awards: None

Publications:

1. Elias, P.M., Park, H.D., Patterson, A.E., Lutzner, M.A., and Wetzel, B.K.: Osmium tetroxide-zinc iodide staining of Golgi elements and surface coats of hydras. J. Ultrastruct. Res. 40: 87-102, 1972
2. Wetzel, B.K., Erickson, B.W., Jr., and Levis, W.R.: The need for positive identification of leukocytes examined by scanning electron microscopy. In Scanning Electron Microscopy/1973. pp. 535-542. (Johari, Om. Ed.), IIT Research Institute, Chicago, Illinois, 1973

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3647

Title: The Fine Surface Morphology, Ultrastructure, and Chemistry of Normal and Diseased Leukocytes During Development, in the Circulation, and in Sites of Leukocytic Infiltrates.

Principal Investigator: Bruce K. Wetzel, Ph.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To define the surface ultrastructure of leukocytes and clarify the physiology of normal and leukemic cells; also, to determine the origin and nature of granulocyte inclusions as indices of development, function, and fate.

Approach:

Buffy coat cells from the peripheral blood of normal humans were diluted in media with autologous serum, allowed to settle on cover slips at 38°C for periods of two minutes to two days, and fixed in situ by the addition of an equal volume of 6% aldehyde. The washed coverslips were stained with Giemsa, photographed as wet mounts, then critically dried and prepared for SEM. In this way, individual cells previously identified by light microscopy (LM) were examined by SEM. In some cases phytohemagglutinin was included in the medium. SEM studies have been initiated on leukocytes from bone marrow, exudates, macerated lymphoid tissues, tissue culture, and patients with leukemia. Representative yields of fixed leukocyte suspensions were deposited and washed on nucleopore filters, trapped in a thin film of water, quick frozen, and lyophilized for SEM. Some additional specimens of normal and leukemic leukocytes from bone marrow, peripheral blood, and tissue culture have been prepared for transmission electron microscopy (TEM) with a variety of fixatives.

Progress: Positive identification of leukocytes by independent means was shown to be absolutely essential for SEM studies, due to the surprising variation in shape and fine surface detail among cells of each series and the remarkable resemblance among many forms of leukocytes from different series. Unequivocal recognition of cells in scanning electron micrographs as members of each leukocyte series, lymphocytes undergoing blastogenesis, dying cells, platelets, megakaryocytes, and myeloid and erythroid precursors has been accomplished by means of our mapping procedures. In addition, we have discovered and described rapid and transient changes in leukocyte form and surface features during adherence and in response to temperature shifts. The surface forms of cells in suspension are under investigation, and initial efforts to monitor cell movement and fixation changes by LM have shown the feasibility of this approach. Significance for Cancer Research (NCP Objective 4 Approach 1) SEM study of buffy coat cells offers a novel and informative view of these cells and provides new bases for comparison with leukemic cells. The key roles of lymphocytes and macrophages in immune responses to malignant growth and therapy are largely dependent on cell surface phenomena and cell interaction--both uniquely amenable to study by SEM and ancillary methods. The origin and nature of leukocyte granules must be known to understand the development of normal and leukemic granulocytes.

Level of Effort: Professional Man-Years: 0.5

Serial No. NCI-3648
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Study of structure and function of abnormal cells in the
Sezary syndrome, Mycosis Fungoides and related Lymphomas

Previous Serial Number: NCI-3617 (Part of)

Principal Investigator: Marvin A. Lutzner, M.D.

Other Investigators: None

Cooperating Units: M. Prunieras, Laboratoire de Recherches. sur les Tumeurs
de la Peau Humaine, Fondation Ad. de Rothschild, 29 rue
Manin, Paris 19e, France; I. Emerit, Laboratoire de
Cytogenetique, Faculte de Medecine, Paris 6e, France;
G. Flandrin, Laboratoire de Cytologie, Institut de
Recherches sur les Leucemies, Hopital St. Louis, Paris
10e, France; C. Grupper and R. Durepaire, Service de
Dermatologie, Fondation Ad. de Rothschild, 29 rue Manin,
Paris 19e, France.

Man Years (July 1, 1972 through June 30, 1973)

Total:	1.0
Professional:	1.0
Other:	0

Project Description:

Objectives:

To study structure, function and oncological significance of the characteristic cell of the Sezary syndrome, and to study similar parameters in malignant cells of mycosis fungoides and related cancers.

The Objective and Approach of the National Cancer Plan that this project most closely supports is 4.2: Biology of cells capable of forming cancer and interactions which host cells - precancerous metabolic and morphologic lesions.

Methods Employed:

1. Patients sought with Sezary syndrome and with mycosis fungoides. Specimens of blood, marrow, skin node, etc. will be obtained.

study.

2. Tissue cells and buffy coat preparations will be Feulgen-stained and examined with a cytophotometer in order to detect presence of polyploid cells.
3. Tissue biopsies and buffy coat pellets will be examined with the electron microscope for morphologic identification of characteristic cells.
4. Chromosome analysis of abnormal tissue, marrow and blood cells will be made in order to determine if chromosomes are normal or abnormal in number and structure.
5. Immunologic techniques will be used to determine whether Sezary cells belong to either B- or T-lymphocyte class.

Major Findings:

1. Two patients with Sezary syndrome have been identified by finding typical cells in blood with electron microscope. Both patients had polyploid cells in peripheral blood as identified by cytophotometry, and both had blood cells in culture with abnormal chromosome number, and "marker" chromosomes. Mitosis occurs in culture only with addition of PHA, indicating lymphocyte nature of cells. Immunological determinations show Sezary cells to be devoid of immunoglobulin at cell surface, indication of possible T-cell origin. Also, 2 patients with typical mycosis fungoides were studied cytophotometrically and cytogenetically and preliminary results show presence of polyploid cells. No marker chromosomes have yet been found. Other mycosis fungoides patients are being studied.
2. Two patients, at first considered to have Sezary syndrome, were found to have cells with abnormal nuclei which are not as convoluted as the nuclei of Sezary cells, were smaller in size, were normal with cytophotometric methods, and rather than high diploidy exhibited, pseudodiploidy or hyperdiploidy. We called this the "small cell variant" syndrome. Two additional patients with "small cell variant" have been identified and their cells have been studied immunologically to determine whether or not they are in the B- or T-lymphocyte class. The cells do not have surface immunoglobulins, do form E-rosettes, and react with a specific anti-T antibody. They therefore appear to be T cells.

Significance to Cancer Research:

Chronic lymphocyte leukemia has been recently found by others to be a B-lymphocyte malignancy. Evidence is accumulating by us and others that Sezary syndrome may be a T-lymphocyte malignancy. If this is so, different approaches, both chemotherapeutic and immunological might be taken with this syndrome.

Proposed Course of Project:

To study additional patients and continue cytogenetic and immunologic investigations. To study additional parameters of structure and function in an attempt to improve diagnosis of this form of cancer and to gain further insight into the biology of these unusual cancer cells and the etiology of their malignant transformation.

Further immunological studies will be done to determine the nature of these cells and also to study their lymphokine products.

Honors and Awards: None

Publications:

1. Lutzner, M.A., Emerit, I., Durepaire, R., Flandrin, G., Grupper, C., and Prunieras, M.: Cytogenetic, cytophotometric and ultrastructural study of large cerebriform cells of the Sezary syndrome and description of a small cell variant. J. Natl. Cancer Inst. (In press).
2. Lutzner, M.A., Ermit, I., Grupper, C., Durepaire, R., Flandrin, G., and Prunieras, M.: Etudes sur les Hematodermies II. Cytophotometrie et Cytogenetique de la cellules de Sezary. Bull. Soc. Fr. Derm. Syph. (In press).

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3648

Title: Study of structure and function of abnormal cells in the Sezary Syndrome, Mycosis Fungoides and Related Lymphomas.

Principal Investigator: Marvin A. Lutzner, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To characterize the structure and function of the abnormal cells in the T cell leukemias.

Approach: To study structure, function and oncological significance of the characteristic cell of the Sezary syndrome, and to study similar parameters in malignant cells of mycosis fungoides and related cancers. (1) Patients sought with Sezary syndrome and with mycosis fungoides. Specimens of blood, marrow, skin node, etc. will be obtained for study; (2) Tissue cells and buffy coat preparations will be Feulgen-stained and examined with a cytophotometer in order to detect presence of polyploid cells; (3) Tissue biopsies and buffy coat pellets will be examined with the electron microscope for morphologic identification of characteristic cells; (4) Chromosome analysis of abnormal tissue, marrow and blood cells will be made in order to determine if chromosomes are normal or abnormal in number and structure; and (5) Immunologic techniques will be used to determine whether Sezary cells belong to either B- or T-lymphocyte class.

Progress: Employing ultrastructural cytophotometric and cytogenetic methods, it has been found that there are two types of abnormal circulating cells occurring in patients with the Sezary erythroderma. First, the big type with a convoluted nucleus, about tetraploid DNA values, and near-tetraploid chromosome counts; and second a small cell variant with an indented irregular nucleus diploid DNA values and a pseudo or hyperdiploid chromosome count. In addition, it has been determined that these large and small cells have no surface immunoglobulins and, therefore, are not B lymphocytes, in contrast to CLL cells, which are predominantly B lymphocytes. In addition, these cells form E-rosettes and react to anti-T antibody, both specific markers for T-lymphocytes.

Significance for Cancer Research (NCP Objective 4 Approach 2)

Chronic lymphocyte leukemia has been recently found to be a B-lymphocyte malignancy. Evidence is accumulating that Sezary syndrome may be a T-lymphocyte malignancy. If this is so, different approaches, both chemotherapeutic and immunological might be taken with this syndrome.

Level of Effort: Professional Man-Years: 1.0

Serial No. NCI-3649
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Enzymatic Control Mechanisms in Melanogenesis

Previous Serial Number: Part of the work has been under NCI-3637

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1972 through June 30, 1973)

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives:

To investigate the control mechanisms involved in regulating melanogenesis in a variety of normal and malignant tissues.

Methods Employed:

Tyrosinase and other enzymic activities are investigated both by spectrophotometric and radioactive assay methodology. Tissues are dissected immediately before use in 0.1 M phosphate buffer, homogenized and fractionated into subcellular fractions by means of density gradient centrifugation and differential centrifugation. Samples are then incubated with the appropriate substrates and controls. The production of melanin and other reaction products can be followed spectrophotometrically or by liquid scintillation counting of the newly formed radioactive melanin.

Major Findings:

Since only one enzyme (tyrosinase) is essential for melanin biosynthesis, it is a unique system for the study of enzymatic control mechanisms in normal and malignant tissues. We have examined the status of enzymatic control of melanogenesis and have found that tyrosinase is the enzyme responsible for melanin synthesis in both murine and avian pigmentary

systems, and not peroxidase, as has been suggested by some workers. We have found that tyrosinase in normal murine pigmentation is controlled, at least at the post-translational level, by a heat-labile, diffusible, low molecular weight inhibitor. On the contrary, tyrosinase in murine melanomas has been reported to be controlled by a heat-stable, non-diffusible inhibitor. In the albino mice, tyrosinase is produced, contrary to previous assumption, but is merely inhibited and never activated for normal melanization. This infers that the c (albino) locus in mice is a regulator, rather than a structural locus, and is probably analogous to human albinism. It has always been a subject of dispute how tyrosinase, which can be easily demonstrated to be present in an active configuration in the endoplasmic reticulum and Golgi apparatus, is inactivated in vivo in mammalian systems and subsequently activated once in situ in the melanosome. In amphibians, the mechanism seems to be proteolytic activation in mammalian and avian systems however, proteolysis has no function in this regard. It seems very likely that in the latter two systems, tyrosinase inhibitor accounts for the inactivation and subsequent activation of the enzyme.

Significance to Cancer Research:

The objective of the National Cancer Plan that this project most closely supports is 3.1 - to study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning. In view of the very obvious differences noted between inhibitors of tyrosinase in normal and melanoma tissues, we feel that at least one of the primary differences between the melanogenic capabilities of these two tissues has been revealed. The fact that these controls are operational at the post-translational level is informative; whether other levels of cellular control mechanisms are affected by carcinogenesis remains to be investigated.

Proposed Course of Project:

Control mechanisms over melanogenesis in normal and malignant melanoma tissues will continue to be investigated. The characteristics of enzymic activity and control in subcellular fractions is being studied. This project will be expanded to include characterization of similar metabolisms in normal and malignant human tissues.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3649

Title: Enzymatic Control Mechanisms in Melanogenesis

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To investigate the control mechanisms involved in regulation of melanogenesis in a variety of tissues of normal and cancerous origin.

Approach: Tyrosinase and other enzymic activities are investigated both by spectrophotometric and radioactive assay methodology. Tissues are dissected immediately before use in 0.1 M phosphate buffer, homogenized and fractionated into subcellular components by means of density gradient and differential centrifugation. Samples are then incubated with the appropriate substrates and controls after treatments known to alter enzymic activity. The production of melanin and other reaction products can be followed spectrophotometrically or by liquid scintillation counting of the newly formed radioactive products.

Progress: We have examined the status of enzymatic control of melanogenesis and have found that tyrosinase is the enzyme responsible for melanin synthesis in both murine and avian pigmentary systems, and not peroxidase as has been suggested by some workers. We have found that tyrosinase in normal murine pigmentation is controlled, at least at the post-translational level, by a heat-stable, non-diffusible inhibitor. In the albino mouse, tyrosinase is produced, contrary to prevailing assumptions, but is merely inhibited and never activated for normal melanogenesis. This infers that the albino locus is a regulator locus in mice and is probably analogous to human albinism.

It has always been a subject of debate how tyrosinase, which is easily demonstrated in endoplasmic reticulum and Golgi regions, is inactivated and subsequently activated in situ in the melanosome, and this inhibitor is a likely

Significance for Cancer Research (NCP Objective 3 Approach 1) answer.

In view of the very obvious differences noted between inhibitors of tyrosinase in normal and melanoma tissues, we feel that these control differences reveal one of the basic variations between melanogenesis in normal and malignant tissues. We are characterizing tyrosinase inhibition in subcellular fractions, and intend to expand the project to include similar studies on melanogenic capabilities in normal and malignant human tissues.

Level of Effort: Professional Man-Years: 0.3

Serial No. NCI-3650
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemical Characterization of Mammalian Melanosomes

Previous Serial Number: Part of this work has been under NCI-3637

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1972 through June 30, 1973)

Total:	0.5
Professional:	0.5
Other:	0.0

Project Description:

Objectives:

To characterize the structural and enzymatic composition of melanin granules throughout their development in vivo, both in normal and abnormal pigmentary systems.

Methods Employed:

Our research has been aimed primarily at characterizing the chemical composition of melanosomes in the mouse, both in the normal black mouse as well as in melanoma tissue. Eyes are excised and homogenized in 0.25 M sucrose, and the melanosomes then isolated and purified by means of an extensive series of differential centrifugations. Granules thus isolated are solubilized in either 0.1 M sodium decyl sulfate or 8 M urea overnight at 80°C, then examined with the aid of polyacrylamide gel electrophoresis. Computer analysis of the resulting protein separations allows the calculation of various physio-chemical parameters of these macromolecules, including molecular weight, molecular size, valence and several other physical constants that are most useful in the comparisons of various proteins.

Major Findings:

The melanosome in normal mouse tissue is composed of seven proteins, six of which are loosely bound and easily extracted. These probably con-

stitute the proteins of the limiting membrane of the organelle. The seventh protein is of lower molecular weight and is tightly bound to the granule, and probably constitutes the structural, fibrillar protein; it appears to be the protein which complexes with the melanin polymer. In addition, this latter protein seems likely to be tyrosinase (if this is the case, tyrosinase would have both structural and enzymatic properties, an unusual occurrence in biological systems). Melanosomal proteins from melanoma tissues vary from those of normal tissue. Several of the high molecular weight proteins seem to be missing completely from these granules, which are also structurally distinguishable from normal granules by electron microscopy. Preliminary results of analyses of granules isolated from three other pigmentary systems (beige mouse, amphibians, and chick) indicate that each contains several proteins in common, but also several unique proteins as well.

Significance to Cancer Research:

The objective and approach of the National Cancer Plan that this project most closely supports is 3.1, to study the nature and modification of the precancerous state and determine mechanisms accounting for high degrees of stability of cell functioning. The observations concerning the aberrant biochemical characteristics of the melanoma melanosome indicate that the control of melanogenesis in malignant melanoma is in some manner affected by carcinogenesis. Melanomas are unusual among cancers since they do not result in complete differentiation of the affected melanocyte, but allow this cell's specialization, i.e. melanogenesis, to continue. These results indicate that although melanogenesis takes place, its metabolic pattern is abnormal.

Proposed Course of Project:

Since there are indications that metabolic controls over melanogenesis are aberrant in murine melanomas, our research is ultimately intended to examine human melanomas, and (assuming melanosome formation is also affected there) attempt to characterize the level on which these disorienting control mechanisms operate. It is hoped that further insight into such controls, be they at the level of replication, transcription, translation, or post-translation, will provide clues as to the level on which carcinogenic information is expressed.

Honors and Awards: None

Publications:

1. Hearing, V.J. and Lutzner, M.A.: Mammalian melanosomes: characterization by polyacrylamide gel electrophoresis, Yale J. Biol. Med., 1973 (In press)

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3650

Title: Biochemical Characterization of Mammalian Melanosomes

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To characterize the structural and enzymatic composition of melanin granules throughout their development in vivo, both in normal and abnormal pigmentary systems.

Approach: Pigmented tissues are excised and homogenized in 0.25 M sucrose, and the melanosomes then isolated and purified by means of an extensive series of differential centrifugations. Granules thus isolated are solubilized in either 0.1 M sodium decyl sulfate or 8 M urea overnight at 80° C, then examined using polyacrylamide gel electrophoresis. Computer analysis of the resulting protein separations allows the calculation of various physico-chemical parameters of these macromolecules, including molecular weight, molecular size, valence and several other physical constants that are most useful in the comparisons of various proteins.

Progress: The melanosome in normal mouse tissue is composed of seven proteins, six of which are loosely bound and easily extracted. These probably constitute the proteins of the limiting membrane of the organelle. The seventh protein is of lower molecular weight and is tightly bound to the granule and probably constitutes the structural, fibrillar protein; it appears to be the protein which complexes with the melanin polymer. In addition, this latter protein seems likely to be tyrosinase, the enzyme which catalyzed melanin formation, and thus this protein might have both structural and enzymatic functions. Melanosomal proteins from melanoma tissues vary from those of normal tissue. Several of the high molecular weight proteins seem to be missing from these granules, which are structurally distinguishable from normal granules, by electron microscopy.

Significance for Cancer Research (NCP Objective 3 Approach 1)

The observations concerning the aberrant biochemical characteristics of the melanoma melanosome indicate that the control of melanogenesis in malignant melanoma is in some manner affected by carcinogenesis. Our research is intended to examine human melanomas and attempt to characterize the level on which these disorienting control mechanisms are operating. It is hoped that further insight into the controls mentioned, be they at the level of replication, etc., will provide clues as to the level on which carcinogenic information is expressed.

Level of Effort: Professional Man-Years:

0.5

Serial No. NCI-3651
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Melanogenesis and Immune Response in Human Melanoma

Previous Serial Number: None

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Other Investigators: Richard Edelson, M.D.
Richard Smith, M.D.
Gary Peck, M.D.

Cooperating Units: NCI, Immunology Branch

Man Years (July 1, 1972 through June 30, 1973)

Total: 0.2
Professional: 0.2
Other: 0.0

Project Description:

Objectives:

1. To characterize melanogenic capabilities of human melanomas and nevi.
2. To culture melanocytes from human melanomas as target cells for immune response studies.

Methods Employed:

1. Tissues selected for examination are dissected out and fixed immediately in glutaraldehyde, then osmium, followed by dehydration, embedding, sectioning, and staining by conventional procedures. Histochemical staining for tissues is also carried out by published electron microscopic techniques.
2. Histocytes and lymphocytes are identified on the basis of surface receptor sites both in paraffin sections and in culture, allowing the differentiation between B cells, T cells and histiocytes. Samples dissociated by trypsin are then separated from the noncellular components by filtration and the intact cells then plated out and cultured on RPMI 1640 culture medium with 10% fetal calf serum.

Major Findings:

Nevus cells have been successfully cultured for the first time and have survived 3 months. These cells contained specific differentiation antigens. Superficial malignant melanomas are infiltrated by macrophages and T cells, while a large congenital nevus was found to be infiltrated by B cells only, and a deep melanoma contained neither type of cell. EM histochemistry demonstrated that both the superficial (pigmented) and the deep (nonpigmented) melanoma tissue contained tyrosinase, the enzyme responsible for melanin formation, but that in the deep tumor this enzyme seems to be suppressed from acting in vivo.

Significance to Cancer Research:

The objective and approach of the National Cancer plan that this project most closely supports is 4.1: Investigative the biology of cells already capable of forming cancer to determine the relationship of their growth and interaction with the host response. It is important to culture melanoma cells so that properly controlled experiments can be carried out regarding their abnormal metabolisms. One such obvious difference is the different degrees of melanogenesis expressed in these tissues; another is the varying surface antigenic properties of these cells.

Proposed Course of Project:

Although the study is just underway, we are preparing to establish cultures of both melanotic and amelanotic melanomas; to characterize their aberrant melanogenic capabilities (in particular to determine the method of tyrosinase inhibition functioning in the amelanotic strain). In addition, we are most interested in pursuing research aimed at determining the unique antigenic properties of the melanocytes, which might be critical to immunotherapy research on malignant melanoma.

Honors and Awards: None

Publications: None

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3651

Title: Melanogenesis and Immune Response in Human Melanoma

Principal Investigator: Vincent J. Hearing, Jr., Ph.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To characterize the melanogenic capabilities of human melanomas and nevi; and to culture melanocytes from human melanomas as target cells for immune response studies.

Approach: Tissues selected for examination are dissected out and fixed immediately in glutaraldehyde, then osmium, followed by dehydration, embedding, sectioning and staining by conventional procedures. Histochemical staining for tissues is also carried out by published electron microscopic techniques. Histiocytes and lymphocytes are identified on the basis of surface receptor sites, both in paraffin sections and in culture, allowing the differentiation between B cells, T cells and histiocytes. Samples dissociated by trypsin are then separated from the noncellular components by filtration and the intact cells then plated and cultured in RPMI 1640 culture media with 10% fetal calf serum.

Progress: Nevus cells have been successfully cultured for the first time and have survived 3 months. These cells contained specific differentiation antigens. Superficial malignant melanomas are infiltrated by macrophages and T cells, while a large congenital nevus was found to be infiltrated by B cells only, and a deep melanoma contained neither type of cell. EM histochemistry demonstrated that both the superficial (pigmented) and the deep (nonpigmented) melanoma tissue contained tyrosinase, the enzyme responsible for melanin formation, but that in the deep tumor this enzyme is suppressed from acting in vivo.

Significance for Cancer Research (NCP Objective 4 Approach 1)

It is important to culture melanoma cells so that properly controlled experiments can be carried out regarding their abnormal metabolisms. One such obvious difference is the different degrees of melanogenesis expressed in these tissues; another is the varying surface antigenic properties of these cells. Our research is aimed at characterizing this tyrosinase inhibition, and determining the unique antigenic properties of the melanocytes, which might be critical to immunotherapy research on malignant melanoma.

Level of Effort: Professional Man-Years: 0.2

Serial No. NCI-3652
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Mechanisms of Cellular Immunity in Man

Previous Serial Number: None (encompasses NCI-3639 and NCI-3607 in past)

Principal Investigator: William R. Levis, M.D.

Other Investigators: A.E. Miller, M.D., Bruce K. Wetzel, Ph.D.

Cooperating Units: None

Man Years (July 1, 1972 through June 30, 1973)

Total:	2.6
Professional:	1.6
Other	1.0

Project Description:

Objectives:

- 1) To investigate the mechanisms concerned with the control of mitosis, differentiation, function, and potentialities of normal and pathologic cells, with particular reference to the lymphocyte and cells of the lymphoreticular system.
- 2) To investigate the functional and morphological interactions between lymphocytes and macrophages and among different populations of lymphocytes during in vitro immune responses related to delayed hypersensitivity, homograft reactions, and cell mediated allergic contact dermatitis.
- 3) To detect, characterize, and isolate biologically active substances, including soluble mediators of cellular immunity, produced in stimulated and unstimulated human leukocyte cultures. To study their effects on supporting, augmenting, and inhibiting blastogenesis, cell division, modulation, and differentiation in vitro.
- 4) To develop an in vitro system for studying allergic contact dermatitis in humans including a method of monitoring immunotherapy of skin cancers with DNCB (see NCI-3641).
- 5) To develop a useful and sensitive histocompatibility test in humans.

- 6) To develop an in vitro model for the study of homograft rejection in humans.
- 7) To develop an in vitro system for studying immune reactivity to various skin cancers. The Objective and Approach of the National Cancer Plan that this project most closely supports is 3.1: Nature and modification of precancerous state and determination of mechanisms accounting for high degrees of stability of cell functioning.

Material:

- 1) Lymphocytes and other peripheral blood cells are obtained from hospitalized patients and outpatients
- 2) Keratome strips of human skin
- 3) Excised tumors of human skin

Methods Employed:

- 1) Human peripheral blood lymphocytes are cultured in vitro and stimulated to transform into large blastoid cells by factors such as phytohemagglutinin, antigens, and foreign tissue. This phenomenon of blastogenesis is evaluated by the morphologic and biochemical changes which the lymphocytes undergo. The mechanisms by which these blastogenic factors act and the culture conditions required for blastogenesis are determined.
- 2) Human peripheral blood leukocytes are separated into a glass-adherent cell population, composed largely of macrophages, and into a population consisting of small lymphocytes. By recombining such populations obtained from the same and from different individuals, the function of each population is determined. Interactions and contacts between cells are examined by light and scanning electron microscopic techniques, including methods for observing the same cells by both modalities.
- 3) A method has been developed for freeze storing leukocytes for subsequent studies of the blastogenic response to various antigens and mitogens.
- 4) A method has been developed for adapting the dinitrochlorobenzene contact sensitization test to the in vitro lymphocyte transformation system.

Major Findings:

- 1) Methods for obtaining 'purified' lymphocytes, glass-adherent mononuclear cells, and a population containing both cell types from human peripheral blood have been developed and analyzed. We have improved and utilized a very rapid and simple 5-minute centrifugation method using heparinized whole blood for obtaining a population of mononuclear

leukocytes (lymphocytes and monocytes) with very few contaminating granulocytes. This mononuclear leukocyte population contains the two cell types required for the blastogenic response and provides cultures capable of responding with maximal responses to specific and non-specific blastogenic factors. Parameters affecting the millipore filter assay technique for measuring tritiated thymidine incorporation into DNA in leukocyte cultures have been studied and analyzed.

- 2) Human epidermal cells induce blastogenesis in allogeneic leukocytes. Human epidermal cells did not induce blastogenesis in autologous leukocytes nor in the leukocytes of a mixed leukocyte HL-A identical sibling pair. These results indicate that epidermal induced leukocyte blastogenesis is a measure of histocompatibility in humans comparable to a one - way mixed leukocyte reaction.
- 3) A method has been developed which permits positive light microscopic identification of individual leukocytes in a heterogeneous population by Giemsa staining while preserving delicate surface features for subsequent scanning electron microscopy of the same cells. (see NCI-3647)
- 4) Isolation and partial characterization of a factor released by glass adherent phagocytic cells which is an essential requirement for the blastogenic response of lymphocytes to specific antigens, leukocytes, and phytohemagglutinin.
- 5) Isolation of a factor released in cultures containing lymphocytes of subjects sensitized to DNCB, that will induce blastogenesis in leukocyte cultures from nonsensitized subjects.
- 6) Characterization of the abnormal blastogenic responses of lymphocytes from patients with chronic lymphocytic leukemia.
- 7) Development of a method for monitoring the degree of sensitivity to DNCB in vitro during in vivo contact sensitization. This in vitro technique expands the usefulness of DNCB for the evaluation and study of cell mediated immunity, and offers a method for monitoring DNCB immunotherapy, (see NCI-3641), an approach for the study of tolerance, and a controlled system for the study of the many antigen-released mediators of cellular immunity in man. Similar approaches with other contact allergens may offer a safe diagnostic test for allergic contact dermatitis.

Significance to Bio-Medical Research and the Program of the Institute:

The lymphocyte is a cell involved in immune reactions which may be operative in the immune response to neoplastic tissue. Our studies concerned with blastogenesis and the control of cell division and differentiation of the lymphocyte may elucidate this cell's normal and abnormal functions in the immune response involved in the host's defense against cancer. These studies should also provide information concerning the control of cell differentiation and division.

Proposed Course of Project:

Continuation of research as indicated in the foregoing.

Honors and Awards: None

Publications:

- 1) Levis, W.R. and Robbins, J.H. Methods for obtaining 'purified' lymphocytes, glass-adherent mononuclear cells, and a population containing both cell types from human peripheral blood. Blood 40: 77-89, 1972.
- 2) Levis, W.R., and Miller, A.E.: Leucocyte/skin cultures as a measure of histocompatibility in man. Lancet 2: 357-360, 1972.
- 3) Robbins, J.H., and Levis, W.R.: Inherent inability of chronic lymphocytic leukemia lymphocytes to respond to phytohemagglutinin. Int. Arch. Allergy Appl. Immunol. 43: 845-858, 1972.
- 4) Miller, A.E., and Levis, W.R.: Lymphocyte Transformation During DNCB Contact Sensitization: An in vitro - in vivo Evaluation of the Primary Immune Response in Man. J. Clin. Invest. (In press).

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 3652

Title:

Studies on Mechanisms of Cellular Immunity in Man

Principal Investigator: William R. Levis, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: A better understanding of cell mediated immunity in man. Particularly, normal or abnormal immunologic mechanisms involved in epithelial and lymphoid malignancies, autoimmune disease, homograft rejection and allergic contact

Approach : dermatitis.

Basic studies on the function and correlative morphology of lymphocytes and macrophages involved in a blastogenic response to specific antigen and foreign tissue. Identification and characterization of soluble mediators produced in stimulated and unstimulated human leukocyte cultures. Characterization of the abnormal blastogenic responses found in certain diseases, particularly lymphoid malignancies. The necessity of both lymphocytes and macrophages in blastogenic responses in vitro has been elucidated and the lymphocyte has been identified as the cell which determines the immunologic specificity of the blastogenic response to antigens. A soluble mediator, released by macrophages, has been found to be an essential requirement for the blastogenic response to antigens and foreign tissue. This factor has been isolated and partially characterized.

Progress: Isolation and study of human epidermal cells and their interactions with leukocytes in vitro has resulted in a histocompatibility test in humans and a potential in vitro homograft model. Characterization of the abnormal blastogenic responses of lymphocytes from patients with chronic lymphocytic leukemia. The successful adaptation of the in vitro lymphocyte transformation technique to the study of dinitrochlorobenzene (DNCB) contact sensitivity has provided a controlled system for the study of antigen-released mediators in man. Because DNCB is a primary immunogen the in vitro technique has provided a method of following the kinetics of a primary and anamnestic immune response in man without altering host reactivity by recurrent challenge. The technique has also provided a method for monitoring patients on DNCB immunotherapy.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Better understanding of cell mediated immunity in man should lead to more rational manipulation of the immune system in favor of the host against incipient or already developed malignancies.

Level of Effort: Professional Man-Years: 1.6

Serial No. NCI-3653
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Identification of Thymus-derived Lymphocytes, Bone Marrow-derived Lymphocytes, and Macrophages in Cutaneous Lesions

Previous Serial Number: None

Principal Investigator: Marvin A. Lutzner, M.D.

Other Investigators: Richard Edelson, M.D., Gary Peck, M.D., Richard Smith, M.D., Michael Frank, M.D. and Ira Green, M.D.

Cooperating Units: NCI, Immunology Branch; NIAID, Immunology Branch and LCI

Man Years (July 1, 1972 through June 30, 1973)

Total: 1.0
Professional: 1.0
Other: 0

Project Description:

Objectives:

- 1) To accurately identify the subpopulations of mononuclear cells in cutaneous infiltrates.
- 2) To correlate the tissue section findings with in vitro cytotoxicity assays.
- 3) To utilize the findings from #1 and #2 in aiding the reralational choice of therapeutic modalities.

Methods Employed:

- 1) Tissues to be studied are sectioned in a cryostat, layered with sheep erythrocytes containing on their surfaces moieties which will specifically interact with receptors on mononuclear cells, rinsed to remove nonadherent cells, fixed in glutaraldehyde, and stained with H and E to be viewed by light microscopy.
- 2) Target cells (melanocytes or epidermal cells) are grown in tissue culture, incubated with serum or lymphocytes, and assayed for cytotoxicity by trypan blue dye exclusion.

- 3) Depending on the tissue section and in vitro findings, a wide range of therapeutic manipulations are attempted. In human disease, cytotoxic drugs more effective against the population identified is chosen. In experimental disease, other agents such as specific antisera against T or B cells is used.

Major Findings:

For the first time, T cells, B cells, and histiocytes have been successfully distinguished from one another in human lesions. Also it has been possible to show that tissue-fixed macrophages lack the receptor for the activated third component of complement found on monocytes.

In several patients with melanomas, T cells were identified infiltrating their lesions. In a patient with a large congenital nevus excised for prophylaxis against melanoma, B cells were identified in large aggregates - suggesting a B cell mediated immune response against these nevus cells.

T cells were found to be the predominant cell type in cutaneous lupus erythematosus lesions, lichen planus, mycosis fungoides, and the Sezary Syndrome.

Significance to Cancer Research:

The Objective and Approach of the National Cancer Plan that this project most closely supports is 4.1: Investigate the biology of cells already capable of forming cancer to determine the relationship of their growth and interaction with the host response.

By identifying the roles of mononuclear cells in defense against non-lymphoid cancer and accurately characterizing the types of lymphoid cells causing various lymphomas, a more rational approach to the treatment and prevention of these diseases is possible.

Proposed Course of Project:

We hope to characterize a broad spectrum of cutaneous disease so that the underlying pathologic processes will be better understood. This work will involve the study both of patients and experimental diseases.

Honors and Awards: None

Publications:

Edelson R, Smith R, Frank M, and Green I. Identification of Subpopulations of Mononuclear Cells in Cutaneous Infiltrates. J. Invest. Dermatol. (In press), 1973.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3653

Title: Identification of T lymphocytes, B lymphocytes, and histiocytes in human lesions.

Principal Investigator: Marvin A. Lutzner, M.D.

Section:

Lab/Branch: Dermatology Branch

Goal: Precise identification of inflammatory cells in lesions.

Approach: Frozen sections of tissue are layered with sheep erythrocytes containing on their surfaces moieties which will specifically interact with receptors on mononuclear cells. Histiocytes have receptor for IgG antibody and B cells have receptors for the third component of complement.

Progress: T cells, B cells, and histiocytes have been accurately identified in normal lymphoid tissue and in a broad spectrum of cutaneous disease.

Significance for Cancer Research (NCP Objective 4 Approach 1)

By identifying these cells in carcinoma lesions, rational manipulation of these processes may be possible.

Level of Effort: Professional Man-Years: 1.0

Serial No. NCI-3654
1. Dermatology Branch
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The development and application of approaches and methods of scanning electron microscopy (SEM) in studies of soft biological tissues.

Previous Serial Number: None

Principal Investigator: Bruce Wetzell, Ph.D.

Other Investigators: Seymour H. Wollman, M.D., W. Jansma, Ph.D., Robert Buscho, M.D., Raphael Dolin, M.D., Grace B. Cannon, Ph.D., and Artrice Bader, Ph.D.

Cooperating Units: Laboratory of Physiology, NCI; Laboratory of Infectious Diseases, NIAID; Johns Hopkins University, Baltimore, Md.; Immunology Department, Litton Bionetics, Bethesda, Md.; and Viral Biology Branch, NCI

Man Years (July 1, 1972 through June 30, 1973)

Total:	2.5
Professional:	0.5
Other:	2.0

Project Description:

Objectives:

To discover and develop new methods and interpretive criteria for the application of SEM to a wide variety of soft biological tissues, and thereby capitalize on the unique capabilities of this instrument in providing improved survey and sampling as an adjunct to other studies, an unparalleled three-dimensional, experiential view of cell form and surface phenomena, and an unusual potential for cytochemical studies.

Methods Employed:

The following tissues have been isolated, fixed, and processed with critical drying procedures for SEM:

- a) Thyroid glands were excised from rats hypophysectomized 24 hours previously without stimulation and at various times after intravenous thyrotropin (TSH) administration. The lobes were sliced with a razor blade, then immersed in aldehyde fixative, and processed

for SEM.

- b) A variety of tissue culture cells which had been grown on cover-slips were fixed in situ, stained, mapped, and photographed without drying by light microscopy, and then processed appropriately for SEM.
- c) Explants of human fetal jejunal mucosa which had been maintained in organ culture for periods of 15 minutes to five days were fixed and processed for SEM. Parallel specimens infected with Herpes simplex virus were also prepared.
- d) Adult S. mansoni, S. japonicum, and S. hematobium were excised from the mesenteric veins of experimental animals, washed, fixed, and processed for SEM.
- e) Samples of chick skin from embryos of varying age and samples cultured for varying periods with and without excessive vitamin A were fixed and processed for SEM. Skin biopsies from a patient with Darier's disease and from normal individuals were also prepared for SEM.

Major Findings:

- a) The apical pseudopods of thyroid follicular epithelium provide a dramatic and relatively controlled example of the way in which a simple surface fold of membrane and cytoplasm (apparently free of microtubules, microfilaments and other internal organelles associated with cell motility or shape) can accomplish an exquisitely complex maneuver to engulf and retract droplets of extracellular material (the luminal colloid). By noting the sequential appearance of new forms after stimulation the following schema is suggested which could account for this process: 1) small ridges appear among the apical microvilli, usually near the cell margins; 2) these ridges enlarge and flatten into rounded sheets which generally parallel the cell surface and overlie the microvilli; 3) the edges of these sheets extend as flaps and curl upward; 4) the flaps and edges continue to extend and curl inward until they overlap, enclosing some of the colloid in a tube or a more rounded structure - probably with a single pore persisting momentarily at the tip; 5) the overlapping flaps fuse and the pore closes and fuses to form a droplet within the pseudopod; 6) the pseudopod assumes a spherical shape and retracts into the cell; 7) finally, a regular array of apical microvilli reappears on the dome of the pseudopod as it subsides. Evidence for lateral branching of the flaps, the development of internal septa and the appearance of further flaps and later forms on the distal portions of rounded, retracting pseudopods could account for the formation of multiple droplets observed within single pseudopods. This interpretation is consistent with TEM and LM observations, but most of these mechanisms are poorly described or misrepresented in published accounts.
- b) Methods which enable scanning microscopy of cells previously stained,

identified, and mapped by light microscopy, have proven useful in studies of a wide variety of cells in tissue culture, (chick embryo) cells, fibroblast lines, melanomas, fibrosarcomas, myosarcomas, renal carcinomas, and cells from pleural effusions of breast cancer patients. Distinctive surface morphology has been found in a few cell lines, but considerable variation has characterized most cultures. Some of this morphological variation is due to asynchronous cell division as cells tend to round up in mitosis. However, preliminary evidence from positively identified cells, mapped after staining their nuclei and chromosomes with Feulgen, methyl green-pyronin, orcein, or Giemsa reveals that rounded cells in interphase may be common and that division figures can sometimes occur in unremarkable flattened cells.

- c) The SEM has proven invaluable in surveying large areas of multiple specimens and drastically reducing sampling error. Difficulties encountered in locating representative LM and TEM fields of sparsely occurring phenomena underscore the usefulness of SEM survey procedures. For example, small efflorescences of elongated microvilli were characteristically found on cultured explants of human fetal jejunum except in the latest, most degenerate specimens, yet they were very rarely captured in vertical section for TEM. These efflorescences were not seen on fragments from a single sample of jejunum which had been placed directly into fixative. Apical swelling of the epithelial cells at sites of cytoplasmic yolk or glycogen was a constant feature of the control explants for the first several days of culture, and pronounced swelling of some areas of the intercellular space was characteristic of control explants cultured two days or longer. Only occasional, scattered surface blebs and moderate sloughing of the cells were noted up through four days in uninfected cultures.

Explants inoculated with Herpes Simplex virus and cultured for two days showed numerous surface blebs and extensive sloughing of cells which remained clumped in the crypts of the explant; the epithelium, however, generally maintained its integrity. Despite an intensive search no virus particles were found in the intact epithelium at this stage by TEM, but massive production of virus particles was commonly seen in the clumps of sloughed cells and occasionally seen in small pair of cells in the submucosa. Further TEM search for virus particles in the epithelium of older cultures revealed a few examples at three days and common occurrence beyond four days. Retraction of the villi and rounding up of the explants were accelerated in the Herpes-infected specimens.

- d) The fine surface morphology of adult S. mansoni has been documented with particular emphasis on the form and distribution of sensory receptors. The variety, number, and focal concentrations of these receptors have been grossly underestimated due to poor preparations in earlier SEM studies and the poor sampling of other methods. Preliminary results have suggested similar distribution of sensory endings in S. japonicum and S. hematobium although their tegumental sur-

faces (previously undescribed) differed markedly from that of S. Mansoni.

e) See Project Report #NCI-3630.

Significance to Cancer Research:

SEM reveals fine surface structure with unparalleled three-dimensionality, depth of field, and sampling capability. As this approach is adapted to biological problems through experience with diverse tissues, cellular form, surface details and interactions can be better interpreted in normal and malignant tissues, as well as in experimental systems which help define immune responses, differentiation, and other processes fundamental to oncogenesis and its control.

In particular, the further delineation of normal thyroid form and function bears on our understanding of hormonal balance in health and disease and provides further basis for comparison with tumors of this gland. The tissue culture studies directly elucidate new aspects of tumor cell biology and transformation. The studies of intestinal explants represent a direct approach to the course of viral infections which in some instances play a major role in malignant transformation. Methods and interpretations developed in the SEM studies of Schistosoma tegument have proven useful in the above studies, and control of Schistosomiasis is thought to bear on the incidence of intestinal neoplasms in endemic populations.

The morphology of cancerous and precancerous skin lesions as compared with normal skin bears on both the understanding and the diagnosis of the disease. Also, keratinizing epithelia provides an excellent model of cellular differentiation, and the skin is an especially involved in immune reactions of interest in the study and treatment of cancer.

Proposed Course of Project:

To further develop and refine techniques of specimen preparation, analysis, and interpretation for SEM will be the goal of continuing work with a wide variety of tissues, experimental approaches, and instrumentation. Special emphasis will be accorded a search for novel cytochemical methods which are uniquely possible with this tool. Most of our attention will be shifted to tissue culture systems for these studies. Further collaboration in SEM, TEM and cytochemical studies of skin is under way with investigators in this branch and elsewhere.

Honors and Awards: None

Publications:

1. Wetzel, B.K., Buscho, R., and Dolin, R: Scanning microscopy of human intestinal explants in organ culture. In 30th Annual Proceedings of the Electron Microscopy Society of America. 396, 397, (Arceneaux, C.J., Ed.). Claitor's Publishing Division, Baton Rouge, Louisiana, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-3654

Title: The Development and Application of Methods in Scanning Electron Microscopy (SEM) in the Study of Soft Biological Tissues.

Principal Investigator: Bruce K. Wetzel, Ph.D.

Section:

Lab/Branch: Dermatology Branch

Goal: To discover and develop new methods and interpretive criteria in the biological applications of SEM.

Approach :

A wide range of different tissues have been prepared, fixed, and processed with critical drying methods for SEM, including (a) thyroid glands from rats hypophysectomized 24 hours previously with and without thyrotropin (TSH) administration; (b) a variety of tissue culture cell lines grown on coverslips, fixed in situ stained and mapped by light microscopy; and (c) explants of human fetal jejunal mucosa which had been maintained in organ culture for periods of 15 minutes to 5 days with and without infection by Herpes virus; parallel TEM was done on all specimens to verify and extend the findings.

Progress:(a) The discrete initiation of macropinocytosis in this tissue in response to TSH has provided clear insight into the mechanics of this process; the eruption of overlapping flaps which engulf the luminal colloid, fuse, and retract into the cell has been unequivocally documented by SEM: (b) Among a variety of neoplastic, non-neoplastic, and transforming cell lines compared under various conditions a few showed generally distinctive surface morphology, and all comprised heterogeneous populations; initial studies of positively identified cells have suggested that surface features may provide neither necessary nor sufficient evidence for the recognition of mitotic cells; and (c) The excellent survey and sampling capabilities of SEM have ensured an accurate TEM analysis of this culture system and of the impact of Herpes infection.

Significance for Cancer Research (NCP Objective 3 Approach 5) Through experience with diverse tissues new SEM approaches can be developed which reveal for the first time important features of cell form, surface detail, and cell interactions in normal and malignant tissues, and in experimental studies of immune responses, differentiation, and other processes fundamental to oncogenesis and its control. Specifically, these findings clarify the response to TSH and bear on studies of thyroid tumors; new aspects of tumor cell biology and transformation are seen; and viral infections, suspect in malignant change, are Level of Effort: Professional Man-Years: well displayed.

0.5

ANNUAL REPORT OF THE METABOLISM BRANCH
SUMMARY OF SIGNIFICANT ACTIVITIES
NATIONAL CANCER INSTITUTE

July 1, 1972 through June 30, 1973

The clinical research program of the Metabolism Branch is directed toward two major goals. The first is to define host factors that result in a high incidence of neoplasia. In this area, a broad range of immunological investigations are carried out in patients with malignancy, in patients with immune deficiency diseases and a high incidence of neoplasia as well as in experimental animal systems to determine the nature of the immune processes that are of importance in the surveillance against neoplastic cells and to define the nature of defects in the immunological system that result in an increased incidence of neoplasia. Major efforts in this area are also directed toward developing techniques for the detection of circulating tumor associated antigens that may be of value in the diagnosis of malignancy. The second major goal of the Branch is to determine the physiological and biochemical effects that a tumor produces on the metabolism of the host. Both patients with neoplastic disease as well as those with non-neoplastic disorders that facilitate the development of techniques for the study of cell membranes, homeostatic mechanisms and metabolic derangements of these control mechanisms are being investigated.

Detection of Circulating Tumor Associated Antigens

A significant effort of the Metabolism Branch over the past year was directed toward developing techniques of value in the diagnosis of different forms of malignancy and for the detection of persisting neoplastic cells in patients receiving therapy for neoplastic disease. New double antibody radioimmunoassay techniques have been established for α -fetoprotein human chorionic gonadotropin, IgG, IgE, IgA, and IgM. α -fetoprotein values over 40 ng/ml were considered abnormal since the serum α -fetoprotein concentration of all of the 130 control individuals over one year of age studied were below this level. Seventy-three percent of the 130 patients with hepatocellular carcinoma from the USA, Africa, or the Orient studied had an elevated α -fetoprotein level with a range in serum concentration from 44 to over 5×10^6 ng/ml. An elevated serum α -fetoprotein level was also very common in patients with testicular teratomas being present in 80% of the 79 patients studied. It should be noted that in over 75% of the cases with testicular tumors and an elevated level the α -fetoprotein concentration was over 40 ng/ml but less than 3000 ng/ml, the lower limit of sensitivity of conventional assay techniques. Thus, these tumors would have been missed if conventional assays had been used.

Elevated α -fetoprotein levels were also noted in a lower frequency in association with other types of tumors. Twenty-two percent of the patients with pancreatic cancer, 17% of the patients with gastric cancer, 4.6% of the patients with colonic cancer, and 7% of the patients with lung cancer had serum α -fetoprotein levels over 40 ng/ml. In contrast to these positive

findings in patients with cancer virtually none of the patients with non-hepatic, chronic diseases had α -fetoprotein levels over 40 ng/ml. Thus, there were only two patients with minimally elevated levels in the 300 patients with an array of chronic diseases studied. It should be noted that physiological elevations of α -fetoprotein are present in pregnant women and in infants under 1 year of age. In addition, some patients with infectious hepatitis, subacute hepatic necrosis and a few patients with cirrhosis of the liver had positive values. Thus, the radioimmunoassay for α -fetoprotein is of value in the diagnosis of cancer in that the serum concentration is elevated in the majority of patients with hepatic and testicular tumors and in some patients with other malignancies but is only very rarely elevated in patients with chronic non-malignant non-hepatic disease. The test is of even greater value in following the efficacy of therapy of these tumors. The detection of α -fetoprotein in the serum of treated patients reflecting the persistence of a few tumor cells was possible with the radioimmunoassay when α -fetoprotein was undetectable as assessed by conventional tests.

It should be noted that the presence of cancer was detected much more frequently when multiple oncofetal markers were studied than when a single marker was utilized. This was especially evident with gastrointestinal tumors when carcinoembryonic and α -fetoprotein were assayed and with testicular and hepatic tumors when human chronic gonadotropin and α -fetoprotein were assessed.

It should be noted that many patients with testicular tumors had an elevation of the two tumor markers human chronic gonadotropin and α -fetoprotein. In some of these patients there was a discordant behavior with human chronic gonadotropin or α -fetoprotein remaining elevated following therapy. This observation suggests that the cells of origin of these proteins are different and that determination of both proteins in the serum of patients undergoing therapy should be performed in order to monitor the response of the different cell types. This use of multiple sensitive assays for tumor markers is of special importance in monitoring the efficacy of therapy since all patients with even modest elevations of either of these markers following treatment had a recurrence of their tumor.

Immunodeficiency Diseases and Malignancy

Patients with immunodeficiency disease and a high incidence of neoplasia are being studied with an ultimate goal of repairing the immunological defect in these patients and of gaining insights into the various immunological processes required for an effective surveillance system against cancer in man. An array of patients with three categories of immune deficiency due to diverse pathophysiological mechanisms are being studied. In the first group exemplified by patients with ataxia telangiectasia, congenital and acquired hypogammaglobulinemia, chronic lymphocytic leukemia, and thymoma and hypogammaglobulinemia there is a defect in the ability to synthesize immunoglobulins and lymphocytes, the effectors of the immune system. In the second type of disorder, exemplified by patients with the Wiskott-Aldrich syndrome, there are defects in antigen processing and recognition, abnormalities in the afferent limb of immunity. Finally, in the third category of disorders,

exemplified by patients with intestinal lymphangiectasia or by patients with Hodgkin's disease with protein and lymphocyte loss into the gastrointestinal tract, there is a short survival of immunoglobulins and lymphocytes that leads to the immune deficiency. All three of the categories of immune deficiency are associated with an increased incidence of malignancy. Eighteen of 110 patients with immunodeficiency disease studied at the National Institutes of Health have developed neoplasms over the past seven years of observation. Special studies that have been carried out with these patients and controls include an analysis of the pathways of cellular differentiation, cellular interaction and cellular biosynthesis that together make up the normal immune response. Studies of the B cells involved in circulating immunity include the study of lymphocyte cell surface receptors for complement, an analysis of lymphocyte cell surface immunoglobulins that function as a membrane bound receptor for antigen, as well as the study of the killer function of B lymphocytes as detected by in vitro antibody directed lymphocyte mediated cytotoxicity. Finally, B lymphocyte function has been assessed by determining the ability of the lymphocytes to synthesize immunoglobulins in vitro and secrete them into the culture media. These secreted immunoglobulins were detected by double antibody radioimmunoassays for IgA, IgM, IgG, and IgE. Thymus derived lymphocyte function was defined by determining the proliferative response of these cells to mitogen, by assaying production of lymphokines such as macrophage migration inhibitory factor, lymphotoxin and chemotactic factor for monocytes. In addition, T-lymphocyte killer cell function was tested in phytohemagglutinin activated killing of ^{51}Cr labeled target cells in vitro.

The study of the immunologic function of patients with ataxia telangiectasia was extended. Ataxia telangiectasia is an inherited multi-system disorder characterized by cerebellar ataxia, oculocutaneous telangiectasia, an increased frequency of infections and a 10% incidence of neoplasia. Patients with this syndrome have an array of abnormalities of both humoral and cellular immune systems. Considering the humoral system, over 70% of the patients lack both serum and secretory IgA and IgE. In addition, the lymphocytes of these patients cultured in vitro for seven days in the presence or absence of mitogens do not synthesize IgA molecules whereas the lymphocytes of normal individuals synthesize 200 to 1000 ng of IgA per 2×10^6 mononuclear cells in culture. Patients with ataxia telangiectasia do, however, have normal numbers of B cells that bear IgA molecules in their cell membrane. Thus, these patients appear to have a defect in the terminal differentiation of these IgA bearing B cells into IgA secreting plasma cells. In the cellular immune system the patients with ataxia telangiectasia responded poorly to skin tests antigens had defects in in vitro lymphocyte transformation and had an absent thymus or a thymus that was embryonic in appearance. This abnormality in thymic development led to the view that the basic defect in patients with ataxia telangiectasia is a defect in the interaction between entodermal, and mesodermal germ line elements that were shown by Auerbach to be required for normal thymic differentiation. We have tested the possibility that such a postulated defect in organ differentiation would lead to the persistent production of fetal proteins including oncofetal serum proteins such as α -fetoprotein by these patients. All of the 35 patients with ataxia telangiectasia studied had markedly elevated levels of this fetal protein

(44 to 2800 ng/ml) whereas none of the 55 patients with other immunodeficiency syndromes nor siblings or parents of patients with ataxia telangiectasia manifested this abnormality. Thus, ataxia telangiectasia is unique among the immunodeficiency diseases in the persistent production of this fetal protein. This supports the hypothesis that the basic defect in this disorder is an abnormality in tissue differentiation due to a defect in the interaction between entodermal and mesodermal elements, an interaction that is required for normal tissue differentiation. Such a disorder in tissue differentiation may also contribute to the very high tumor incidence in patients with this disorder.

Patients with chronic lymphocytic leukemia were shown to have lymphocytes that were markedly defective in in vitro proliferation to mitogens, in target cell killing and in lymphokine secretion following stimulation with non-specific mitogens or antigens. The predominant lymphocyte in these patients was a B cell bearing a homogenous immunoglobulin usually 8S IgM on their surface. The lymphocytes of these patients synthesized IgM in vitro at a normal or increased rate but did not synthesize IgG, IgA or IgE normally. Patients with this syndrome have reduced levels of normal immunoglobulins especially IgG and IgE in the serum. This disorder appears to be a malignancy of the B cell lymphocyte system with the excessive proliferation of a single clone of these cells. The production of other normal clones of B cells and the differentiation of these clones into plasma cells is reduced.

The study of the nature of immune defects of patients with the Wiskott-Aldrich syndrome, the prototype of the second category of immune deficiency has continued. The Wiskott-Aldrich syndrome is an x-linked immunodeficiency disorder with a very high incidence of infections and of reticuloendothelial malignancy. Patients with this syndrome have pronounced defects in both humoral and cellular immunity characterized by clinical anergy and poor specific antibody production. Intensive study of these patients has revealed, however, that much of the effector arm of their immune system is intact. They have normal numbers of circulating T and B lymphocytes as detected by immunofluorescence or microcytotoxicity assays with specific antisera. They produce immunoglobulins at normal or elevated rates. Their lymphocytes have normal proliferative responses in vitro to nonspecific mitogens and they produce lymphokines such as macrophage migration inhibitory factor (MIF), lymphotoxin and chemotactic factor for monocytes normally. In addition, the killer function of their B lymphocytes is normal as detected by in vitro antibody directed lymphocyte mediated cytotoxicity, and their T lymphocyte killer cells are normal as tested in PHA activated killing of ^{51}Cr -labeled target cells in vitro. In spite of these normal in vitro tests these patients lack adequate specific humoral and cellular immune responses both in vitro and in vivo which supports our view that the immunodeficiency of this disorder is the result of a defect in afferent limb of immunity perhaps a defect in antigen recognition or processing. The occurrence of immunoglobulin paraproteins or lymphoid malignancies in a high proportion of these patients suggest that the normal regulatory mechanisms which control proliferation of immunocompetent cells is also defective in this syndrome, probably as a result of the same cellular defect resulting in immunodeficiency.

Studies of the immunologic defects of the third category of immune deficiency exemplified by intestinal lymphangiectasia have been continued. Intestinal lymphangiectasia is a disease characterized by a disorder of lymphatic channel development leading to dilatation of the lymphatic channels of the small bowel, the hallmark morphologic lesion of this disease. These patients lose both serum proteins and lymphocytes into the gastrointestinal tract as a consequence of the abnormal intestinal lymphatics. As a result of the lymphocyte loss there is a profound lymphocyte depletion and depressed cellular immunity. In previous studies we demonstrated that there was a depletion of the circulating lymphocyte population which mediates cellular lymphoid functions. In these studies, patients with intestinal lymphangiectasia were shown to have depressed in vitro blastogenic responses to both specific and nonspecific stimuli. We postulated that this qualitative defect in the lymphocyte pool of intestinal lymphangiectasia patients was due to the selective depletion of long lived recirculating T-lymphocytes by intestinal loss. Both the pattern of recirculation of these cells through lymphatic channels and their long survival makes them especially susceptible to depletion by the process of bulk loss of lymph into the gastrointestinal tract. In recent studies, we have further substantiated this hypothesis by showing that the residual lymphocyte population in the blood of patients with intestinal lymphangiectasia is relatively enriched in B cells and depleted in T cells. Specifically we have demonstrated that the percentage of lymphocytes bearing cell membrane immunoglobulin markers (i.e., B cells) is substantially increased. The selective depletion of lymphocytes which mediate cell-mediated immunity may explain the high incidence of reticulo-endothelial malignancies which we have observed in this condition.

Patients with sprue and gastrointestinal diseases were also investigated in the overall study of the relationship of immunological disorders in the pathogenesis of human disease. An in vitro model of gluten sensitive enteropathy was developed in order to analyze the role of immunologic factors in this disease. Jejunal biopsy specimens were maintained in organ culture for as long as 48 hours. During this period cellular alkaline phosphatase activity increased in specimens from normal individuals probably because of cellular maturation in vitro. Initial alkaline phosphatase levels in specimens from patients with gluten-sensitive enteropathy were low and underwent a significant increase during culture. However, if gliadin was added to the culture medium the increase in alkaline phosphatase was not seen. This inhibition of enzyme activity increase in culture by gliadin constitutes an in vitro model of gluten sensitive enteropathy.

To resolve the question of whether gliadin has direct toxicity on mucosal cells or acts through an endogenous effector mechanism the above in vitro model of gluten sensitive enteropathy was utilized. The cultured behavior of jejunal specimens from patients with gluten sensitive enteropathy in remission was compared with specimens from the same patients following gluten challenge, that is with gluten sensitive enteropathy in exacerbation. Specimens from patients with remitted disease underwent alkaline phosphatase increases in the presence or absence of gliadin. In contrast, specimens from those patients with active disease underwent enzyme increase in the absence but not the presence of gliadin. If specimens from patients with active

disease were cultured with specimens of patients with remitted disease in the presence of gliadin, the remitted tissue now behaved like active tissue; alkaline phosphatase increase was inhibited. Finally, cell-free homogenates of tissue from active patients also caused the inhibition of alkaline phosphatase increases in the presence of gliadin. These findings indicated that the toxic potential of gliadin in vitro was manifested only in specimens from patients with active disease or if tissue extracts of active disease patients were present. This represents compelling evidence that endogenous mechanisms (such as the immunologic systems) must be activated before gliadin could exert a toxic effect.

A large group of patients with dermatitis herpetiformis was studied. This disease is characterized by vesicular skin eruption controlled with sulfones. There is an associated villous atrophy which responds to gluten restriction. It was shown that approximately 90% of patients with gluten sensitive enteropathy and approximately 60-65% of patients with dermatitis herpetiformis bear the HL-A8 histocompatibility type. For dermatitis herpetiformis this finding has been confirmed in three separate studies involving 30 patients, each of this disease. This similarity of HL-A8 type in gluten sensitive enteropathy and dermatitis herpetiformis provides a genetic association between the two diseases.

Patients with dermatitis herpetiformis underwent studies of bowel function and intestinal immunoglobulin synthesis as estimated using ^{14}C -leucine incorporation into immunoglobulin by biopsy specimens in short-term culture. It was found that increases in IgA synthesis also accompanied histologic evidence of bowel disease. Villous atrophy was found exclusively in those patients bearing HL-A8 whereas those not bearing HL-A8 were generally free of bowel disease. This study supports the concept that HL-A8 and bowel disease in both dermatitis herpetiformis and gluten sensitive enteropathy are related. The relationship may be through an abnormal immune response gene (Ir gene) which causes patients to mount immunologic responses to gluten proteins. This is suggested by the fact that Ir genes in experimental animals are linked to histocompatibility genes. Alternatively, histocompatibility genes in both diseases may determine abnormal binding sites on epithelial cells which then is associated with binding of gluten on cell surfaces, immunologic responses, and immune destruction of cells.

Bilirubin Metabolism

Studies of hepatic physiology in normal individuals and patients with liver disease have been expanded during the past year. Measurement of hepatic bilirubin clearance, using the radiobilirubin technique devised in this laboratory has proved to be a valuable indication of liver function in patients with various disease states. In particular, measurement of hepatic bilirubin clearance has been shown to permit a useful and highly accurate classification of patients with unconjugated hyperbilirubinemia into those with predominantly hepatic pathology and those with "pre-hepatic" abnormalities. Thus, measurement of bilirubin clearance may ultimately be as useful for the evaluation and management of patients with liver disease as measurement of creatinine clearance is for the management of renal disease. In

order to make this measurement more widely accessible to clinicians, a new approach was developed which permits the accurate estimation of bilirubin clearance from measurements of red cell half-life, using ^{51}Cr . This eliminated the need for liquid scintillation counting equipment and large digital computers, which are required by the original technique.

Patients with Gilbert's syndrome have continued to serve as a useful model for the study of organic anion transport by the human liver. In the past year we have demonstrated that the hepatic storage capacity for bilirubin is reduced in patients with Gilbert's syndrome, and that changes in bilirubin storage capacity during drug therapy are at least as important as glucuronyl transferase induction in producing the lowered plasma bilirubin concentration observed during phenobarbital administration in man. Studies of sulfo-bromophthalein (BSP) kinetics revealed that patients with Gilbert's syndrome were a heterogenous population. One third had normal BSP metabolism, one third had a defective BSP uptake, and one third had a defect at a later stage in the BSP transport process. Detailed studies of BSP kinetics in normal volunteers during mild pyrogen-induced fever showed that even minimal increases above normal body temperature produced marked abnormalities of BSP metabolism in the absence of liver disease.

In collaboration with Dr. Paul Plotz (NIAMDD), a simple "artificial liver" for the extraction of protein bound organic anions from blood is under development. Preliminary studies in jaundiced rats show highly efficient removal of both conjugated and unconjugated bilirubin when the system is perfused with whole blood from a surgical arterio-venous shunt. There is no damage to formed elements and no apparent loss of clotting factors during perfusion of this system.

Porphyria Metabolism

A steady state mathematical model of irreversible pathways, using Michaelis-Menten kinetics, has been developed. The equation which describes the steady state pool size of any intermediate in such a pathway has been solved as a function of 5 parameters, the precursor substrate concentration, the $V_{\max}$ of the enzyme which synthesizes and of the enzyme which utilizes the intermediate and the K_m 's of these two enzymes. The model describes the relationship between enzyme levels, inhibitors and other variables and the concentration of intermediates in an irreversible pathway. It allows calculation of how much increase in an enzyme is necessary to overcome a "partial block" in a metabolic pathway. The model was designed to aid in the elucidation of the biochemical consequences of certain inborn errors of metabolism, particularly the porphyrias.

Studies of the basic biochemical defects, clinical characteristics and optimal mode of therapy in patients with acute intermittent porphyria have been continued. The decrease of uroporphyrinogen I synthetase which is known to occur in the liver in acute intermittent porphyria has now also been demonstrated in fibroblasts and circulating red cells from these patients. A method for the measurement of the enzyme in red cells has been developed and it is being used to determine the feasibility of an enzymatic diagnosis of the latent and active disease from a blood sample.

Histidine administration has been shown to lower porphyrin precursor excretion in acute intermittent porphyria and to lower porphyrin excretion in cutanea tarda porphyria. The effects are rapid, of large magnitude and reversible on withdrawal of histidine. The histidine mobilizes large amounts of body zinc for excretion, but administration of large amounts of zinc along with histidine does not reverse the effects on porphyrin or porphyrin precursor excretion. Therefore, it is thought that histidine does not produce these effects through its alterations of zinc metabolism.

A pronounced synergistic effect on the hyperglycemia produced by diazoxide has been demonstrated by the combination of this compound with certain other compounds, i.e., allylisopropylacetamide or certain barbiturates.

New methods have been developed for the isolation and purification of tissue heme. Heme labeling can be demonstrated in vivo using precursor glycine or tricarboxylic acid cycle intermediates. Studies of the effect of tumors, various types of experimental porphyria and other variables on these synthetic pathways are now in progress.

Amino Acid Transport and Biosynthesis

Studies of amino acid transport are being continued in fetal rat calvaria as well as in cultured cells. Amino acid uptake in both isolated tissue and cultured cells were shown to be regulated by two mechanisms both dependent on the intracellular concentration of specific amino acids. These mechanisms suggest feedback inhibition and repression mechanisms. Both cyclic AMP and insulin stimulate amino acid uptake in fetal rat calvaria. Specific amino acids blocked the cyclic AMP but not the insulin stimulation. This finding suggests that cyclic AMP may be producing its stimulation by decreasing the intracellular amino acid concentration whereas insulin has a direct effect on the transport mechanism. In addition the amino acid loading effect is sodium dependent suggesting that cell membranes participate in the processing of the intracellular amino acid signal for regulatory uptake.

Specific and highly sensitive radioisotopic assays for ornithine- δ -trans-aminase and L-pyrroline-5-carboxylate reductase activities have been developed. The assays involve the use of cation exchange chromatography to isolate specific labelled products. Sensitivity in both assays is adequate to quantitate enzyme activities in as few as 10^6 cultured mammalian cells.

Regulation of this proline biosynthetic pathway in a number of cell lines was studied using these assays. Pyrroline-5-carboxylate reductase is very sensitive to proline feedback inhibition. $2 \times 10^{-4}M$ proline inhibits the enzyme activity to 50%. Although this sensitivity to proline inhibition is exhibited by enzyme isolated from all cell lines tested (HTC, CHO-K, CHL, D550), enzyme isolated from rat livers is relatively insensitive to proline inhibition. Proline concentration of $10^{-2}M$ are necessary to produce 50% inhibition. The mechanism for this apparent enzyme alteration are being investigated. The occurrence of isoenzymes or hormonal influences are being considered.

An interesting cell line (CHO-K₁) derived from Chinese hamster ovaries are also being studied. These cells are proline auxotrophs and require proline concentrations of 10^{-4} M to grow. Other epithelial cells from Chinese hamsters (CHL) are prototrophic for proline. Since both glutamate and ornithine may serve as precursors for proline, it was of interest to define the enzymatic defect in CHO-K₁ cells. The formation of proline from precursor glutamate is nil in CHO-K₁ cells. The activity of ornithine- δ -transaminase is about 15% of that found in CHL cells. Double-cloned CHO-K₁ cells have the same activity. The CHO-K₁ enzyme exhibits identical affinities for substrate and heat-inactivation profiles as the CHL enzyme. These findings suggest that the primary defect is in the glutamate pathway but more important that the pathway from ornithine is regulated by intermediates of the glutamate pathway.

Calcium Metabolism

Studies of the effect of diphosphonates in a variety of diseases with ectopic calcifications have been continued. Combining balance and ^{47}Ca kinetic techniques with bone densitometry, we found that the drug decreases rates of turnover and prevents new ectopic calcifications from forming. In addition, the density of bone as well as growth and developmental parameters are unaltered by the drug. These findings support the early use of the drug in children with diseases of ectopic calcifications.

Calcium kinetic data from 10 patients with hypoparathyroidism were compared with data from 15 normals. Using compartmental analysis it was found that parathyroid hormone not only increases bone resorption but also increases the entry of calcium from rapidly turning over compartments into plasma. These findings suggest that parathyroid regulation of calcium transport processes may play a role in the hormone-dependent maintenance of plasma calcium homeostasis.

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2. Metabolism Branch
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PHS-NIH

Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Physiology and Pathophysiology of Plasma Protein Metabolism

Previous Serial Number: SAME

Principal Investigator: Thomas A. Waldmann, M.D.

Other Investigators: Warren Strober, M.D., R. Michael Blaese, M.D., and K. Robert McIntyre, M.D.

Cooperating Units: Laboratory of Cell Biology, NCI

Man Years:

Total: 4
Professional: 2
Others: 2

Project Description:

Objectives: The objectives of the study were to determine the normal rate of synthesis, transport and catabolism of the plasma proteins, especially the immunoglobulins. To determine the physiological factors controlling these metabolic parameters and to determine the pathophysiology of disorders of serum protein metabolism in patients with neoplastic disease and in patients with hereditary disorders of immunoglobulin metabolism. In addition, studies were directed toward developing techniques for the detection of circulating tumor-related antigens and in determining the metabolism of these protein antigens.

Methods Employed: The rates of synthesis, catabolism and distribution of the serum proteins were studied using radioiodinated labeled purified proteins. New double antibody radioimmunoassays were established to detect α -fetoprotein, human chorionic gonadotropin and the immunoglobulins IgG, IgA, IgM, and IgE.

Major Findings: A significant effort over the past year was directed toward developing techniques of value in the diagnosis of different forms of malignancy and for the detection of persisting neoplastic cells in patients receiving therapy for neoplastic disease. New double antibody radioimmunoassay techniques have been established for α -fetoprotein human chorionic gonadotropin, IgG, IgE, IgA, and IgM. α -fetoprotein

values over 40 ng/ml were considered abnormal since the serum α -fetoprotein concentration of all of the 130 control individuals over one year of age studied were below this level. Seventy-three percent of the 130 patients with hepatocellular carcinoma from the USA, Africa, or the Orient studied had an elevated α -fetoprotein level with a range in serum concentration from 44 to over 5×10^6 ng/ml. An elevated serum α -fetoprotein level was also very common in patients with testicular teratomas being present in 80% of the 79 patients studied. It should be noted that in over 75% of the cases with testicular tumors and an elevated level the α -fetoprotein concentration was over 40 ng/ml but less than 3000 ng/ml, the lower limit of sensitivity of conventional assay techniques. Thus, these tumors would have been missed if conventional assays had been used.

Elevated α -fetoprotein levels were also noted in a lower frequency in association with other types of tumors. Twenty-two percent of the patients with pancreatic cancer, 17% of the patients with gastric cancer, 4.6% of the patients with colonic cancer, and 7% of the patients with lung cancer had serum α -fetoprotein levels over 40 ng/ml. In contrast to these positive findings in patients with cancer virtually none of the patients with nonhepatic, chronic diseases had α -fetoprotein levels over 40 ng/ml. Thus, there were only two patients with minimally elevated levels in the 300 patients with an array of chronic diseases studied. It should be noted that physiological elevations of α -fetoprotein are present in pregnant women and in infants under 1 year of age. In addition, some patients with infectious hepatitis, subacute hepatic necrosis and a few patients with cirrhosis of the liver had positive values. Thus, the radioimmunoassay for α -fetoprotein is of value in the diagnosis of cancer in that the serum concentration is elevated in the majority of patients with hepatic and testicular tumors and in some patients with other malignancies but is only very rarely elevated in patients with chronic non-malignant non-hepatic disease. The test is of even greater value in following the efficacy of therapy of these tumors. The detection of α -fetoprotein in the serum of treated patients reflecting the persistence of a few tumor cells was possible with the radioimmunoassay when α -fetoprotein was undetectable as assessed by conventional tests.

It should be noted that the presence of cancer was detected much more frequently when multiple oncofetal markers were studied than when a single marker was utilized. This was especially evident with gastrointestinal tumors when carcinoembryonic and α -fetoprotein were assayed and with testicular and hepatic tumors when human chronic gonadotropin and α -fetoprotein were assessed.

It should be noted that many patients with testicular tumors had an elevation of the two tumor markers human chronic gonadotropin and α -fetoprotein. In some of these patients there was a discordant behavior with human chronic gonadotropin or α -fetoprotein remaining elevated following therapy. This observation suggests that the cells of origin of these proteins are different and that determination of both proteins in the

serum of patients undergoing therapy should be performed in order to monitor the response of the different cell types. This use of multiple sensitive assays for tumor markers is of special importance in monitoring the efficacy of therapy since all patients with even modest elevations of either of these markers following treatment had a recurrence of their tumor.

The study of α -fetoprotein has also provided insights into the basic defect of patients with ataxia telangiectasia. Ataxia telangiectasia is an inherited multi-system disorder characterized by cerebellar ataxia, oculocutaneous telangiectasia, and increased frequency of infections and a 10% incidence of neoplasia. Patients with this syndrome have an array of abnormalities of both humoral and cellular immune systems. Considering the humoral system, over 70% of the patients lack both serum and secretory IgA and IgE. In addition, the lymphocytes of these patients cultured in vitro for seven days in the presence or absence of mitogens do not synthesize IgA molecules whereas the lymphocytes of normal individuals synthesize 200 to 1000 ng of IgA per 2×10^6 mononuclear cells in culture. Patients with ataxia telangiectasia do, however, have normal numbers of B cells that bear IgA molecules in their cell membrane. Thus, these patients appear to have a defect in the terminal differentiation of these IgA bearing B cells into IgA secreting plasma cells. In the cellular immune system the patients with ataxia telangiectasia responded poorly to skin tests antigens had defects in in vitro lymphocyte transformation and had an absent thymus or a thymus that was embryonic in appearance. This abnormality in thymic development led to the view that the basic defect in patients with ataxia telangiectasia is a defect in the interaction between endodermal, and mesodermal germ line elements that were shown by Auerbach to be required for normal thymic differentiation. We have tested the possibility that such a postulated defect in organ differentiation would lead to the persistent production of fetal proteins including oncofetal serum proteins such as α -fetoprotein by these patients. All of the 35 patients with ataxia telangiectasia studied had markedly elevated levels of this fetal protein (44 to 2800 ng/ml) whereas none of the 55 patients with other immunodeficiency syndromes nor siblings or parents of patients with ataxia telangiectasia manifested this abnormality. Thus, ataxia telangiectasia is unique among the immunodeficiency diseases in the persistent production of this fetal protein. This supports the hypothesis that the basic defect in this disorder is an abnormality in tissue differentiation due to a defect in the interaction between endodermal and mesodermal elements, an interaction that is required for normal tissue differentiation. Such a disorder in tissue differentiation may also contribute to the very high tumor incidence in patients with this disorder.

The metabolism of IgE in patients with neoplasia was investigated. Patients with tumors of the B cells (thymic independent lymphocytes) i.e., patients with chronic lymphocytic leukemia, multiple myeloma and macroglobulinemia were shown to have markedly reduced IgE levels due to decreased IgE synthesis. In contrast patients with active

Hodgkin's disease had an elevated IgE level due to increased synthesis. The patients with Hodgkin's disease had disorders of cellular immunity and the elevated IgE levels may be secondary to the lack of adequate numbers of regulatory T cells that normally terminate IgE antibody responses.

The study of diseases associated with protein losing gastroenteropathy was continued. Protein losing gastroenteropathy has been demonstrated in association with 80 diseases. Over the past year this process has been shown to be a significant factor in the hypoalbuminemia of patients with a variety of forms of cancer.

Significance to Biomedical Research: The detection of circulating tumor-related antigens is of significance in the diagnosis of neoplastic disease and is of special relevance in evaluating the activity of the neoplastic disease and determining the efficacy of therapy of these disorders. The studies on immunoglobulin metabolism extend our knowledge of the normal metabolism and physiological control mechanisms that affect immunoglobulin synthesis, transport and catabolism. They are of importance in understanding the pathophysiology of disorders of immunoglobulin levels in patients with disease.

Proposed Course: Studies directed toward understanding the pathways and controlling mechanisms of the synthesis, transport, and catabolism of serum protein will be continued. Increasing emphasis will be placed on the development of new techniques for detection of circulating tumor-related antigens. Studies will also be directed toward defining the synthetic rates and rates of catabolism of such tumor-related antigens.

Honors and Awards

None

Publications

Gill, J., Waldmann, T.A. and Bartter, F.C.: Idiopathic edema: I. The occurrence of hypoalbuminemia and abnormal albumin metabolism in women with unexplained edema. Amer. J. Med. 52: 444-451, 1972.

Jones, E.A. and Waldmann, T.A.: The mechanism of intestinal uptake and transcellular transport of IgG in the neonatal rat. J. Clin. Invest. 51: 2916-2927, 1972.

Langer, T., Strober, W. and Levy, R.I.: The metabolism of low density lipoprotein in familial type II hyperlipoproteinemia. J. Clin. Invest. 51: 1528-1536, 1972.

McFarlin, D.E., Strober, W. and Waldmann, T.A.: Ataxia-telangiectasia. Medicine. 51: 281-314, 1972.

Polmar, S.H., Waldmann, T.A., Balestra, S.T., Jost, M., and Terry, W.D.: Immunoglobulin E in immunologic deficiency diseases: I. Relation of IgE and IgA to respiratory tract disease in isolated IgE deficiency, IgA deficiency and ataxia telangiectasia. J. Clin. Invest. 51: 326-330, 1972.

Polmar, S.H., Waldmann, T.A. and Terry, W.: IgE in immunodeficiency. Amer. J. Pathol. 69: 499-512, 1972.

Waldmann, T.A.: Protein-losing enteropathy and kinetic studies of plasma protein metabolism. In Freeman, L.M. and Blaufox, M.D. (Eds.): Radionucleid Studies of the Gastrointestinal System. New York, Grune and Stratton, 1972, pp. 153-166.

Waldmann, T.A.: Protein-losing enteropathy and kinetic studies of plasma protein metabolism. Sem. Nucl. Med. 2: 251-264, 1972.

Waldmann, T.A. and McIntire, K.R.: Serum alpha-fetoprotein levels in patients with ataxia-telangiectasia. Lancet. 2: 1112-1114, 1972.

Waldmann, T.A., Polmar, S.H., Balestra, S.T., Jost, M.D., Bruce, R.B., and Terry, W.D.: Immunoglobulin E in immunologic deficiency diseases. II. Serum IgE concentration of patients with acquired hypogammaglobulinemia, thymoma and hypogammaglobulinemia, myotonic dystrophy, intestinal lymphangiectasia and the Wiskott-Aldrich syndrome. J. Immunol. 109: 304-310, 1972.

Waldmann, T.A., Strober, W. and Blaese, R.M.: Disorders of immunoglobulin catabolism. In Peeters, H. (Ed.): Protides of the Biological Fluids. Proceedings of the 19th Colloquium. 1971, Brugges, Belgium Pergamon Press, 1972, pp. 425-428.

Waldmann, T.A., Strober, W. and Mogielnicki, R.P.: The renal handling of low molecular weight protein. II. Disorders of serum protein catabolism in patients with tubular proteinuria, nephrosis, or uremia. J. Clin. Invest. 51: 2162-2174, 1972.

Kellermeyer, R.W., Warren, K.S., Waldmann, T.A., Cook, J.A., and Jordan, P.: Serum immunoglobulin concentrations in St. Lucians with schistosomiasis compared with matched uninfected St. Vincetians. J. Infec. Dis. In press.

Polmar, S., Waldmann, T.W. and Terry, W.: A comparison of three radio-immunoassay techniques for the measurement of serum IgE. J. Lab. Clin. Med. In press.

Polmar, S.H., Waldmann, T.A. and Terry, W.D.: Quantitation of IgE: Problems encountered in measurement of low levels of IgE in serum. In Dayton, K. and Ishizaka, K. (Eds.): The Biological Role of the Immunoglobulin E System. In press.

Polmar, S.H., Waldmann, T.A. and Terry, W.D.: The relationship of IgA and IgE deficiency. In Good, R.A. (Ed.): Primary Immunodeficiency Diseases. The National Foundation. In press.

Waldmann, T.A.: Immunoglobulin metabolism in diseases. In Good, R.A. (Ed.): Primary Immunodeficiency Disease. The National Foundation. In press.

Waldmann, T.A.: Protein losing gastroenteropathies. In Bockus, H.L. (Ed.): Gastroenterology, 3rd edition. In press.

Waldmann, T.A.: Protein losing gastroenteropathy. In Wagner, H.N., Jr. (Ed.): Principals of Nuclear Medicine. 2nd Edition. W.B. Saunders. In press.

Waldmann, T.A.: The use of radiolabeled macromolecules in the evaluation of gastrointestinal protein loss in children. In James, A.E. and Wagner, H.N., Jr. (Eds.): Pediatric Nuclear Medicine. In press.

Waldmann, T.A., Strober, W., Blaese, R.M. and Terry, W.: Immunoglobulin metabolism in disease. In Bergsma, D. and Finstead, J. (Eds.): Proceedings of the Second International Workshop of Immunodeficiency Diseases. In press.

Waldmann, T.A., Strober, W. and Mogielnicki, R.P.: The proteinuria of cystinosis: Its pattern and pathogenesis. In Schulman, R. (Ed.): Cystinosis. In press.

Waldmann, T. A., Strober, W. and Mogielnicki, R.P.: The role of the kidney in the metabolism of low molecular weight proteins. Protides of the Biological Fluids. 19th Annual Colloquium, Brugges, Belgium. Pergamon Press. 1971. In press.

Warshaw, A.L., Waldmann, T.A. and Laster, L.: Protein-losing enteropathy and malabsorption in regional enteritis: Cure by limited ileal resection. Ann. Surg. In press.

Wochner, R.D., Drews, G., Strober, W., and Waldmann, T.A.: IgG metabolism in myotonic dystrophy. Proceedings of the Second International Symposium on the Biochemistry of Muscle Disorders. Montreal. In press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-4002(c)

Title: Studies of the Physiology and the Pathophysiology of Plasma Protein Metabolism

Principal Investigator: Thomas A. Waldmann, M.D.

Section: Immunophysiology Section

Lab/Branch: Metabolism

Goal: To determine the pathophysiology of disorders of serum protein synthesis transport and catabolism. In addition to develop immunological tests for the diagnosis of cancer.

Approach The rates of synthesis, catabolism and transport of immunoglobulins and tumor related antigens were studied using radioiodinated purified proteins. The data are analyzed using a multicompartamental model for protein metabolism. In addition new double antibody radioimmunoassays were established to detect α -fetoprotein, human chorionic gonadotropin, and immunoglobulins IgG, IgA, IgM, and IgE. These assays were evaluated as techniques for the diagnosis of different forms of malignancy and for the detection of persisting neoplastic cells in patients receiving therapy for cancer.

Elevated α -fetoprotein levels were detected in 43% of patients with hepatomas, 80% of patients with teratomas of the testes, 22% of patients with pancreatic carcinoma, and 17% of patients with

Progress: gastric cancer. The α -fetoprotein and human chorionic gonadotropin assays have been of special value in following efficacy of therapy of patients with cancer. The detection of the oncofetal markers was possible with the sensitive radioimmunoassay methods used when they were undetectable by conventional tests. All patients with ataxia telangiectasia studied had embryonic thymus glands and elevated α -fetoprotein levels suggesting that the primary disorder in this disease is a defect in organ differentiation.

In other studies it was shown that IgE synthesis was markedly depressed in patients with neoplasms of B lymphocytes (i.e., patients with CLL and myeloma) and increased in patients with Hodgkin's disease.

Significance for Cancer Research (NCP Objective 5 Approach)

The detection of circulating tumor related antigens is of significance in the diagnosis of neoplastic disease and is of special relevance in evaluating the activity of neoplastic disease and determining the efficacy of therapy of these disorders. The studies of immunoglobulin metabolism are of importance in understanding the pathophysiology of disorders of immunoglobulin levels in patients with neoplasia.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-4003(c)

1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Studies of Porphyrin Metabolism in the Tumor-Bearing Host and Porphyria

Previous Serial Number: SAME

Principal Investigator: Donald P. Tschudy, M.D.

Other Investigators: Joel B. Levine, M.D.

Cooperating Units: National Heart and Lung Institute, Experimental Therapeutics Branch

Man Years

Total: 4

Professional: 2

Other: 2

Project Description:

Objectives: To study the mechanisms controlling heme biosynthesis and biochemical and clinical aspects of the porphyrias. To apply the knowledge gained from the above objectives to the study of tumors and tumor host relationships as well as primary diseases of porphyrin metabolism.

Methods Employed: Quantitative enzyme determinations, chemical determination of porphyrins and porphyrin precursors, use of tissue preparations and isotope methods in studying metabolic pathways, and development of new methods for isolation and purification of tissue heme.

Major Findings: Barbiturates and allylisopropylacetamide have been shown to produce a pronounced synergistic effect on the hyperglycemia resulting from diazoxide administration. Barbiturates or allylisopropylacetamide, when given with diazoxide, produce a synergistic effect on the hyperglycemia in both starved and glucose-loaded animals.

The third enzyme of the heme biosynthetic pathway, uroporphyrinogen I synthetase, is known to be decreased in the liver of patients with acute intermittent porphyria. The decrease of this enzyme has now been shown in fibroblasts and red cells of these patients. A method for the measurement of this enzyme in red cells has been developed and it is being used to determine whether acute intermittent porphyria can be diagnosed enzymatically from a sample of red cells. The data indicate that the red cell enzyme measurement can be used to diagnose the disease, but the statistical

details are not yet completed.

A family in which the father has porphyria cutanea tarda and the daughter has erythropoietic protoporphyria is being studied. These 2 diseases have never been reported previously in this genetic relationship.

Increased levels of circulating free thyroxine have been shown to occur in 2 patients during attacks of acute intermittent porphyria. These findings have been accompanied by extensive studies of thyroid physiology and indicate another complex abnormality in this disease.

Histidine administration lowers porphyrin precursor excretion in acute intermittent porphyria and porphyrin excretion in cutanea tarda porphyria. The effects are rapid, of large magnitude and reversible on withdrawal of histidine. Histidine mobilizes large amounts of body zinc for excretion, but administration of large amounts of zinc along with histidine does not reverse the effects on porphyrin or porphyrin precursor excretion. Therefore it is thought that histidine does not produce these effects through its alterations of zinc metabolism.

New methods have been developed for the purification of tissue heme. These methods are being applied to the measurement of overall activity of the heme biosynthetic pathway in liver. The effect of tumors, experimental porphyria and other variables on this pathway is in progress.

Significance to Biomedical Research: Mechanisms of the control of metabolic pathways and the synthesis of enzymes in normal and diseases tissues are being elucidated. Some of the findings have been used for practical application to the diagnosis and treatment of certain types of porphyria. New abnormalities of control mechanisms in tumors and host tissues have been demonstrated and are under current study.

Proposed Course: (A) To continue studies of the factors controlling heme and heme-enzyme synthesis in normal and pathologic tissues. (B) To continue studies of biochemical abnormalities of porphyria in man and experimental animals. (C) To study control mechanisms of heme synthesis in tumors and livers of tumor-bearing hosts.

Honors and Awards

None

Publications

Tschudy, D.P.: Acute intermittent porphyria. In Bergsma, D. (Ed.): Birth Defects Atlas and Compendium. Baltimore, Williams and Wilkins, 1973, pp. 146-147.

Tschudy, D.P.: Coproporphyria. In Bergsma, D. (Ed.): Birth Defects Atlas and Compendium. Baltimore, Williams and Wilkins, 1973, p.302.

Tschudy, D.P.: Erythropoietic porphyria. In Bergsma, D. (Ed.): Birth Defects Atlas and Compendium. Baltimore, Williams and Wilkins, 1973, pp. 387-388.

Tschudy, D.P.: Erythropoietic protoporphyria. In Bergsma, D. (Ed.): Birth Defects Atlas and Compendium. Baltimore, Williams and Wilkins, 1973, pp. 388-389.

Tschudy, D.P.: Variegate porphyria. In Bergsma, D. (Ed.): Birth Defects Atlas and Compendium. Baltimore, Williams and Wilkins, 1973, p. 882.

Stein, J.A., Curl, F.D., Valsamis, M., and Tschudy, D.P.: Abnormal iron and water metabolism in acute intermittent porphyria with new morphologic findings. Am. J. Med. 53: 784-789, 1972.

Tschudy, D.P. and Watson, C.J.: Comments on paper, "Zwei Suchteste fur Porphyrien" by Doss, M. and Schmidt, A. (1972) this j.10 230-231. Zeitschr. Klin. Chem. Biochem. 11: 128-129, 1973.

Tschudy, D.P.: Book Review: The porphyrias: A story of inheritance and environment. by G. Dean, 2nd edition, Philadelphia, J.B. Lippincott., Co. 1971, pp. 176. Am. J. Human Genetics. 25: 220-221, 1973.

Bonkowsky, H.L., Collins, A., Doherty, J. and Tschudy, D.P.: The glucose effect in rat liver. Studies of δ -aminolevulinic acid synthetase and tyrosine aminotransferase. Biochim. Biophys. Acta. In press.

Bonkowsky, H.L., Tschudy, D.P., Collins, A., and Doherty, J.: Control of δ -aminolevulinic acid synthetase and tyrosine aminotransferase in tumors and livers of tumor-bearing hosts. J. Nat. Cancer Inst. In press.

Tschudy, D.P. and Bonkowsky, H.L.: A steady-state model of irreversible enzyme reactions. Molec. Cell. Biochem. In press.

Tschudy, D.P.: Enzyme aspects of acute intermittent porphyria. Molec. Cell. Biochem. In press.

Tschudy, D.P.: Porphyrin metabolism and the porphyrias. In Duncan's Diseases of Metabolism. In press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-4003(c)

Title: Studies of Porphyrin Metabolism in the Tumor-Bearing Host and Porphyrria

Principal Investigator: Donald P. Tschudy, M.D.

Section:

Lab/Branch: Metabolism Branch

Goal: To elucidate the biochemical and clinical aspects of porphyrin metabolism in the porphyrias and tumors and tumor-bearing hosts.

Approach Many of the techniques which relate to studying the effects of variables on metabolic pathways are employed. These include measurement of enzymes, development of new methods for enzyme measurements, measurement of pathway activities by use of isotopic substrates, isolation and measurement of metabolites from tissues, blood and urine and the use of mathematical models of metabolic pathways. Studies are performed in patients and animals.

Progress: Barbiturates and allylisopropylacetamide produce a marked synergistic effect on the hyperglycemia caused by diazoxide. A method for measurement of uroporphyrinogen I synthetase in red cells has been developed and is now being shown to be useful as an enzymatic method for the diagnosis of acute intermittent porphyria. Histidine administration lowers porphyrin and porphyrin precursor excretion in cutanea tarda porphyria and acute intermittent porphyria. New methods have been developed for the purification of tissue heme and the measurement of the heme biosynthetic pathway in liver.

Significance for Cancer Research (NCP Objective 5B Approach 4)

Abnormalities of metabolic control mechanisms are relevant to abnormal growth. We have shown marked abnormalities in the control of heme biosynthesis in tumors and host livers. Our data suggest a circulating repressor substance (for ALA synthetase) which originates in tumors. If such a substance can be measured in blood, it could be used for diagnosis and monitoring treatment. Preliminary efforts in this regard are as yet unsuccessful.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-4004(c)

1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Amino Acid Transport and Amino Acid Biosynthesis

Previous Serial Number: SAME

Principal Investigator: James M. Phang, M.D.

Other Investigators: S. Peter Nissley, M.D. and David L. Valle, M.D.

Cooperating Units: None

Man Years:

Total: 6
Professional: 2
Others: 4

Project Description:

Objectives: Amino acid biosynthesis and amino acid uptake play an important role in cellular growth and metabolism. The mechanisms by which these processes are regulated in normal and malignant cells are poorly understood. This project emphasizes the regulatory interplay between membrane transport of endogenous amino acids and the endogenous biosynthesis of amino acids. This project is relevant to Objective 3, Approaches 1 and 5 of the National Cancer Program.

Methods Employed: We are primarily using animal cells maintained in long-term tissue culture. Cells are cloned to insure a genetically homogenous population. Mutants are isolated by standard techniques. A variety of biochemical techniques have been utilized to study amino acid transport processes and amino acid biosynthesis in cultured cells. Uptake of amino acids may be assessed by using labeled L-amino acids and rates of protein and collagen synthesis can be obtained by incubating cells with L-proline- C^{14} and separation of the radioisotopically incorporated amino acids as proline and hydroxyproline by thin layer chromatography. The enzymes for biosynthesis of proline in the liver are studied using labeled precursors such as L-ornithine and L-glutamic acid and by isolating the products using ion exchange chromatography.

Major Findings: Studies of amino acid transport are being continued in fetal rat calvaria as well as in cultured cells. Amino acid uptake in both isolated tissue and cultured cells were shown to be regulated by two mechanisms both dependent on the intracellular concentration of

specific amino acids. These mechanisms suggest feedback inhibition and repression mechanisms. Both cyclic AMP and insulin stimulate amino acid uptake in fetal rat calvaria. Insulin overcomes the repression mechanism but does not alter the effects of feedback inhibition. Cyclic AMP stimulation appears to override the feedback inhibition mechanism. This finding suggests that cyclic AMP may be producing its stimulation by altering the mass-action effect of intracellular amino acids whereas insulin produces a depression of the transport mechanism. In addition, the amino acid loading effect is sodium dependent suggesting that cell membranes participate in the processing of the intracellular amino acid signal for regulating uptake.

Specific and highly sensitive radioisotopic assays for ornithine- δ -transaminase and L-pyrroline-5-carboxylate reductase activities have been developed. The assays involve the use of cation exchange chromatography to isolate specific labelled products. Sensitivity in both assays is adequate to quantitate enzyme activities in as few as 10^6 cultured mammalian cells.

Regulation of this proline biosynthetic pathway in a number of cell lines was studied using these assays. Pyrroline-5-carboxylate reductase is very sensitive to proline feedback inhibition. $2 \times 10^{-4}M$ proline inhibits the enzyme activity by 50%. Although this sensitivity to proline inhibition is exhibited by enzyme isolated from all cell lines tested (HTC, CHO-K1, CHL, D550), enzymes isolated from rat liver, brain or kidney, are relatively insensitive to proline inhibition. Proline concentration of $10^{-2}M$ are necessary to produce 50% inhibition. The mechanism for this apparent enzyme alteration are being investigated. The occurrence of isoenzymes and substrate or hormonal influences are being considered.

An interesting cell line (CHO-K1) derived from Chinese hamster ovaries are also being studied. These cells are proline auxotrophs and require proline concentrations of 10^{-4} to grow. Other epithelial cells from Chinese hamsters (CHL) are prototrophic for proline. Since both glutamate and ornithine may serve as precursors for proline, it was of interest to define the enzymatic defect in CHO-K1 cells. The formation of proline from precursor glutamate is absent in CHO-K1 cells. In addition, the activity of ornithine- δ -transaminase the first enzyme of the ornithine pathway is about 15% of that found in CHL cells. Double-cloned CHO-K1 cells have the same activity. The CHO-K1 enzyme exhibits identical affinities for substrate and heat-inactivation profiles as the CHL enzyme. These findings suggest that the primary defect is in the glutamate pathway but more important that the pathway from ornithine is regulated by intermediates of the glutamate pathway.

Significance to Biomedical Research: Cell membranes may play an important role in the regulation of cellular physiology. Changes in cell membrane function affects cellular metabolism and cell proliferation. Some of these changes are produced by hormone regulation as a component

of normal control mechanisms. Abnormal changes in membrane function may result in uncontrolled neoplastic cell growth. Investigators have proposed that one of the primary changes produced by malignant transformation may be alterations in membrane function. These workers have shown that a viral transformation of cells can increase rates of sugar and amino acid transport even prior to increases in cell growth rates. Our studies on the regulation of amino acid transport and the interplay between membranes and endogenous biosynthetic mechanisms may elucidate the biochemical mechanisms of membrane function as well as derangements of these functions in malignant cells.

Proposed Course: We are isolating CHO-K1 cells which have reverted to proline prototrophy to characterize the glutamate and ornithine pathways of proline biosynthesis. Using standard protein purification techniques, we are purifying the proline biosynthetic pathways to investigate the interplay between the two pathways. We suspect that a phosphorylation or an adenylation process is involved.

The regulation of transport is being studied in cultured cells. Since our studies suggest that hormonal effects may alter the response to regulation by intracellular amino acids, we are seeking direct evidence of this interaction on a molecular level.

Honors and Awards

None

Publications

Weiss, I.W., Morgan, K. and Phang, J.M.: Cyclic AMP-stimulated transport of amino acids in kidney cortex. J. Biol. Chem. 247: 760-764, 1972.

Weiss, I.W., Harris, S., Downing, S.J., and Phang, J.M.: Microdetermination of phosphorylated intermediates of $\text{Na}^+\text{-K}^+$ adenosine triphosphatase. Anal. Biochem. 46: 453-460, 1972.

Phang, J.M. and Downing, S.J.: Amino acid transport in bone: Stimulation by cyclic AMP. Amer. J. Physiol. 224: 191-196, 1973.

Phang, J.M., Downing, S.J. and Valle, D.: A radioisotopic assay for ornithine- δ -transaminase. Anal. Biochem. In press.

Phang, J.M., Downing, S.J. and Valle, D.: A radioisotopic assay for pyrroline-5-carboxylate reductase. Anal. Biochem. In press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-4004(c)

Title: Studies of Amino Acid Transport and Amino Acid Biosynthesis

Principal Investigator: James M. Phang, M.D.

Section:

Lab/Branch: Metabolism Branch

Goal: To eliminate the regulatory mechanisms for membrane transport and biosynthesis of amino acids in normal and malignant cells.

Approach

Using tissue-culture methods, we are inducing and isolating mutants of mammalian cell lines. We have developed sensitive assays for the enzyme of proline biosynthesis to study the regulatory mechanisms in the cells we have isolated. We are emphasizing the interplay between membrane transport mechanisms and the biosynthetic pathways for supplying amino acids to the cell. Purified enzymes and membrane fractions are studied to define the molecular mechanisms of interactions.

Progress: The amino acid transport mechanism can be regulated by both repression and feedback inhibition. Insulin appears to alter the repression mechanism whereas cyclic AMP changes the feedback inhibition. Both of these hormonal agents override the decreased transport seen with increasing intracellular amino acids.

The two pathways for endogenous proline biosynthesis appear to be regulated. By studying selected mutants, we showed that an intermediate of the glutamate pathway for proline biosynthesis can interact and regulate the ornithine pathway for proline production. The regulation may occur at either the genetic or protein level.

Significance for Cancer Research (NCP Objective 3 Approach 1&5)

The regulation of amino acids may be important in controlling the growth rate of normal and malignant cells. The interaction between a membrane signal and endogenous mechanisms is of special interest in controlling malignant cell growth.

Level of Effort: Professional Man-Years: 2 years

Serial No. NCI-4009(c)

1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: A Study of the Factors Controlling Red Blood Cell Synthesis and Destruction

Previous Serial Number: SAME

Principal Investigator: Paul D. Berk, M.D.

Other Investigators: Bruce F. Scharschmidt, M.D.

Cooperating Units: None

Man Years:

Total: 5
Professional: 5
Others: 0

Project Description:

Objectives: To study the factors influencing rates of red blood cell production and destruction in normal and pathological states in man, the dog and rat, with particular emphasis on the role of ineffective erythropoiesis in the overall body economy of heme. Studies of hepatic heme enzyme turnover and organic anion transport are also conducted as an outgrowth of the use of isotopically labeled bilirubin to quantitate erythropoiesis.

Methods Employed: Erythropoiesis is quantitated by the following techniques: whole blood and red cell volumes are determined with ^{51}Cr -labeled red blood cells; red cell life span is determined directly from either ^{14}C -glycine incorporation or tritiated diisopropylfluorophosphate labeling, or calculated from plasma bilirubin turnover; plasma and red blood cell iron turnovers are calculated from the plasma clearance of ^{59}Fe -labeled transferrin, and subsequent incorporation of radio-iron into circulating erythrocytes. Ineffective erythropoiesis is estimated from the incorporation of ^{14}C label into fecal urobilinogen during the first ten days after IV administration of ^{14}C -glycine, as well as by computer analysis of the specific activity curve of plasma bilirubin following the administration of labeled heme precursors such as glycine and delta-aminolevulinic acid. Bilirubin- ^{14}C and bilirubin- ^3H are prepared biosynthetically from delta-aminolevulinic acid; bilirubin production rate, hepatic bilirubin clearance and storage, and intercompartmental rate constants in the bilirubin excretory pathway are determined by computer analysis of the

plasma radio-bilirubin disappearance curves. An independent estimate of heme turnover is obtained by measurement of carbon monoxide excretion. Hepatic transport of organic anions such as sulfobromophthalein (BSP) and indocyanine green is studied by computer analysis of their respective plasma disappearance curves.

Major Findings: During heme catabolism in vitro, 1 mole of bilirubin and 1 mole of carbon monoxide (CO) are produced for each mole of heme degraded. Since it is generally accepted that this process is the only biological source of CO, it is assumed that measurements of either bilirubin turnover or CO production should be equivalent, and both parameters have been used clinically to estimate hemoglobin catabolism and red cell life span. Recently, additional biological sources of CO have been described, and reported measurements of CO production have appreciably exceeded estimates of bilirubin production in several studies in both man and animals. We therefore performed simultaneous measurements of bilirubin turnover (BRT) and CO production (COP) in 37 individuals including both normal volunteers and patients with up to an 8 fold increase in heme catabolism due to hemolysis. The data were highly correlated ($r=0.99$) and fitted an equation of the form $COP=BRT + \text{constant}$. The magnitude of the difference between COP and BRT, $0.95 \mu\text{M/kg/day}$, was largely attributable to heme degradation and bilirubin synthesis in the liver, which is not entirely reflected in BRT. These studies support the hypothesis that heme catabolism results in the equimolar formation of bilirubin and CO in vivo; indicate that clinical measurements of BRT and COP can be used to accurately measure red cell survival; and indicate that the various recently reported alternate biologic sources of CO must contribute very little to total CO production in man.

We have previously determined that administration of enzyme-inducing drugs such as phenobarbital and glutethimide markedly increases bilirubin clearance and reduces plasma bilirubin levels in patients with Gilbert's syndrome, and produces similar but less dramatic changes in normal volunteers. Such changes have been attributed by others largely to induction of bilirubin UDP glucuronyl transferase. During the past year, compartmental analysis of radiobilirubin and BSP kinetic studies, performed before and after treatment with either phenobarbital or glutethimide in 11 normal controls and 19 patients with Gilbert's syndrome, revealed the following pattern of changes during drug administration: (a) Hepatic bilirubin uptake rate increased to $184 \pm 10\%$ (mean $\pm$ SEM), conjugation to $150 \pm 12\%$ and storage to $240 \pm 23\%$ of baseline in patients with Gilbert's syndrome. Corresponding results in normal volunteers were: uptake = $125 \pm 6\%$, conjugation $91 \pm 10\%$ and storage $159 \pm 20\%$ of baseline. Changes in overall bilirubin clearance far exceeded changes in conjugation rate produced by drug administration, indicating that drug-induced alterations in hepatic bilirubin uptake and storage were at least as important as increased conjugation in lowering plasma bilirubin levels. An appreciable increase in hepatic BSP uptake also occurred as a result of drug administration. In contrast to these effects of low doses of drug ($< 5 \text{ mg/kg}$) in man, we demonstrated that much higher doses (75 mg/kg) of both phenobarbital and glutethimide did induce increased levels of the conjugating enzyme bilirubin UDP glucuronyl transferase in rats.

We have previously reported that administration of phenobarbital and glutethimide produces a fall in the rate of plasma bilirubin turnover to an average of 85% of baseline. In the past year, studies to elucidate the mechanism of this decrease were carried out in 6 normal volunteers and 7 patients with Gilbert's syndrome. Delta amino levulinic acid-4-¹⁴C (ALA-¹⁴C) a specific precursor of hepatic heme, and hence, of bilirubin produced by the liver, and bilirubin-³H were administered simultaneously, and the plasma specific activity curves of bilirubin-³H and bilirubin-¹⁴C (derived in the liver from ALA-¹⁴C) determined. By a mathematical technique known as deconvolution, carried out on a Univac 1108 digital computer, the data were used to calculate the rate of entry to plasma of bilirubin synthesized in the liver. By comparing data obtained before and during drug administration, it became apparent that a decreased flux of bilirubin from liver to plasma during treatment is one reason plasma bilirubin turnover falls with drug administration. Further studies are in progress to determine whether these observations reflect decreased hepatic heme turnover, or more efficient excretion of the bilirubin derived from hepatic hemes directly into bile, without prior passage through the plasma.

Hepatic bilirubin clearance has proved to be a useful indicator of hepatic function. Its determination requires a source of pure radiobilirubin of high specific activity, and radiation counting equipment and digital computers not widely available to clinicians. In contrast, most community hospitals can easily measure the total red cell volume and red cell half-life, using radiochromate. Accordingly a simple equation was derived for the estimation of hepatic bilirubin clearance from radiochromium measurements of red cell volume and half-life. Using this equation, estimates of bilirubin clearance in 21 normal volunteers (0.68 ± 0.05 ml/min/kg; mean $\pm$ SEM), 19 patients with Gilbert's syndrome (0.20 ± 0.02 ml/min/kg) and 2 patients with Crigler-Najjar syndrome (0.011 ± 0.002 ml/min/kg) agreed very well with corresponding values derived from the more complex radiobilirubin technique (0.65 ± 0.04 ; 0.20 ± 0.02 and 0.010 ± 0.003 ml/min/kg, respectively). A linear regression on 61 pairs of data was virtually indistinguishable from the line of identity. This approach should make measurement of hepatic bilirubin clearance more accessible to clinicians outside large research institutions.

Measurements of relative hepatic storage capacity for bilirubin were performed with radiobilirubin in 32 patients with Gilbert's syndrome and 28 normal volunteers. Values in the patient group were consistently reduced (0.35 ± 0.02 mg/mg%/kg) compared with normal controls (0.69 ± 0.05 mg/mg%/kg). The possibility that a reduced bilirubin storage capacity may explain previously reported abnormalities in both uptake and conjugation is currently under investigation. Since storage capacity appears to relate to the quantity of available intrahepatic Y and Z proteins, isolation, purification and development of immunoassays for these materials are currently being pursued.

In other studies mild, pyrogen induced fever was shown to produce abnormal 45 minute BSP retention in 9 of 20 normal volunteers, but a reduced BSP storage capacity and other abnormalities of BSP kinetics in 100% of cases.

In collaboration with Dr. Paul Plotz (NIAMDD), a simple "artificial liver" for the extraction of protein-bound organic anions from blood is under development. Preliminary studies in jaundiced rats show highly efficient removal of both conjugated and unconjugated bilirubin when the system is perfused with whole blood from a surgical arterio-venous shunt. There is no damage to formed blood elements and no apparent loss of clotting factors during perfusion of this system.

Significance to Biomedical Research: A large variety of drugs, including many cancer chemotherapeutic agents, are either detoxified or converted to their active form in the liver. Many are also hepato-toxic. Studies with radio-labeled bilirubin and other studies of organic anion transport performed in this laboratory are intended: (1) to develop new techniques and new quantitative information about normal hepatic function in man; (2) to study the effects of simple drugs on various aspects of hepatic function, developing and using methodology ultimately applicable to studying the effects of cancer chemotherapeutic agents on the liver; (3) to examine various physiologic parameters as possible early indicators of hepato-toxicity and (4) to examine the feasibility of developing an artificial support organ, analogous to the artificial kidney, capable of temporarily sustaining patients with hepatic failure due to drugs and other causes.

Proposed Course: Studies of various aspects of hepatic organic anion transport and drug metabolism in man will be pursued. The basic mechanisms underlying hepatic enzyme induction by drugs will be investigated. Feasibility of the extracorporeal perfusion system for treatment of hepatic failure will be investigated in a variety of animal models.

Honors and Awards

None

Publications

Berk, P.D., Blaschke, T.F., and Waggoner, J.G.: Defective BSP clearance in patients with constitutional hepatic dysfunction (Gilbert's syndrome). Gastroenterology 63:472-481, 1972.

Berk, P.D. and Blaschke, T.F.: Detection of Gilbert's syndrome in patients with hemolysis, using radioactive chromium. Annals of Internal Medicine 77:527-531, 1972.

Blaschke, T.F. and Berk, P.D.: Augmentation of bilirubin UDP-glucuronyl transferase activity in rat liver homogenates by glutethimide. Proceedings of the Society for Experimental Biology and Medicine 140:1315-1318, 1972.

Jones, E.A., Shrager, R., Bloomer, J.R., Berk, P.D., Howe, R.B., and Berlin, N.I.: Quantitative studies of the delivery of hepatic-synthesized bilirubin to plasma utilizing 8-aminolevulinic acid-4-¹⁴C and bilirubin-³H in man. Journal of Clinical Investigation 51:2450-2458, 1972.

Berk, P.D., Berlin, N.I. and Howe, R.B.: Disorders of bilirubin metabolism, in Duncan's Diseases of Metabolism, 7th Edition, P.K. Bondy and L. Rosenberg, Eds. W.B. Saunders and Co., Philadelphia (In press).

Blaschke, T.F., Elin, R.J., Berk, P.D., Song, C.S. and Wolff, S.M.: Effect of induced fever on sulfobromophthalein kinetics in man. Annals of Internal Medicine 78:221-226, 1973.

Berk, P.D., Blaschke, T.F. and Rodkey, F.L.: Effects of phenobarbital and glutethimide on heme turnover, plasma bilirubin turnover and hepatic bilirubin clearance. In Preisig, R. and Paunggartner, G. (Eds.): The Liver: Quantitative Aspects of Structure and Function. Basel, S. Karger 1973, (In press).

Scharschmidt, B.F., and Berk, P.D.: A simple device to facilitate rapid blood sampling in small animals. Proceedings of the Society for Experimental Biology and Medicine (In press).

Berk, P.D., Berlin, N.I., Scharschmidt, B.F. and Blaschke, T.F.: Hepatic bilirubin clearance (C_{BR}) in the differential diagnosis of unconjugated hyperbilirubinemia: Estimation of C_{BR} from studies with radiochromium. Epatologia (In press).

Berlin, N.I. and Berk, P.D.: The biological life of the red blood cell, in The Red Blood Cell, 2nd Ed., D.M. Surgenor, Ed., Academic Press, New York (In press).

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 4009(c)

Title: A Study of the Factors Controlling Red Blood Cell Synthesis and Destruction

Principal Investigator: Paul D. Berk, M.D.

Section:

Lab/Branch: Metabolism Branch

Goal: To develop techniques for assessing multiple diverse aspects of hepatic function quantitatively in man; and to examine both the pharmacologic and toxic effects of drugs on these parameters.

Approach

The kinetics of a number of test substances extracted from plasma by the liver have been studied. Materials studied include exogenous drugs and dyes (e.g. sulfobromophthalein [BSP]) as well as endogenous metabolites such as bilirubin and bile acids. Following intravenous injection of test substances, detailed plasma disappearance curves are obtained. These are analyzed on a large digital computer in terms of a relevant compartmental model, to derive estimates of the rates of biological processes. For bilirubin, this permits calculation of bilirubin clearance, hepatic bilirubin uptake and storage capacity, conjugation rate, bilirubin production rate, and red cell lifespan. Attempts are made to validate model-derived estimates by comparing similar kinetic studies with direct experimental measurement in animals. Effects of drugs on various aspects of hepatic function are determined by performing paired studies during both control and treatment periods.

Progress:

This technique has made possible the first quantitative estimates of several aspects of hepatic function in man, and has permitted more precise characterization of the functional abnormalities in several disease states. It has also led to the recognition that clinical entities such as Gilbert's syndrome may be heterogeneous, resulting from several different kinetic abnormalities. Studies with drugs have demonstrated that agents thought to alter plasma clearances of various metabolites primarily by hepatic microsomal enzyme induction may, in fact, operate by entirely different mechanisms. Information gained from these studies has led to preliminary attempts to develop an artificial liver.

Significance for Cancer Research (NCP Objective ² Approach ²)

Many cancer chemotherapeutic agents are either activated or inactivated by the liver, and many are also severely hepatotoxic. These studies are aimed at improving our knowledge of normal hepatic function, the alterations produced by drugs, and means for minimizing and managing drug-induced toxicity to permit fuller exploitation of chemotherapeutic agents.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-4013(c)

1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Studies of Calcium Metabolism in Malignant Disease and Metabolic Diseases of Bone

Previous Serial Number: SAME

Principal Investigator: James M. Phang, M.D.

Other Investigators: David L. Valle, M.D. and Mones Berman, Ph.D.

Cooperating Units: Mathematical Research Branch, NIAMDD

Man Years:

Total: 4

Professional: 2

Others: 2

Project Description:

Objectives: Osseous metastases with or without accompanying hypercalcemia are a frequent occurrence in neoplastic disease. In many patients the hypercalcemia can be life threatening. This project is designed to investigate physiologic and pathophysiologic regulatory mechanisms and to understand derangements of these mechanisms with malignant processes. This project is relevant to Objective 3, Approach 1, and Objective 7, Approach 2 of the National Cancer Program.

Methods Employed: Serum Ca^{47} disappearance and excretion of Ca^{47} into urine and feces are combined with metabolic balance data in a computer multicompartmental model to give steady state rate constants and numerical values for metabolic parameters. The physiological hypothesis suggested by this analysis are tested in animal and in vitro experiments and by additional studies of calcium metabolism in man. Furthermore, the uptake of Ca^{47} into the blood pool after an oral administered dose is used to define the absorption of calcium from the gastrointestinal tract. By varying the calcium load of the test dose we can define a quantitative relationship between calcium absorption and gastrointestinal luminal concentrations. Where applicable, bone histology, urinary hydroxyproline measurements, bone biopsy and bone densitometry studies are obtained.

Major Findings: Studies of the effect of diphosphonates in a variety of diseases with ectopic calcifications have been continued. Combining

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-4013(c)

Title: Studies of Calcium Metabolism in Malignant Disease and Metabolic Diseases of Bone

Principal Investigator: James M. Phang, M.D.

Section:

Lab/Branch: Metabolism

Goal: To understand the mechanisms of calcium homeostasis and bone metabolism in normal subjects and patients with metabolic and malignant bone disease.

Approach

We use balance and Ca^{47} kinetics to study calcium and bone metabolism. Combining these techniques with bone densitometric measurements and urinary hydroxyproline determinations, we can assess the parameters of bone metabolism.

Progress: Diphosphonates, an analog of pyrophosphate can decrease pathologic calcifications. Bone turnover rates are also decreased by the drug. The beneficial effects occur without retardation of normal growth and development.

Significance for Cancer Research (NCP Objective3&7 Approach1&2)

Hypercalcemia with or without osseous metastases is of frequent occurrence in patients with malignancies. An understanding of both normal and pathophysiology of calcium and bone metabolism may lead to new therapeutic approaches.

Level of Effort: Professional Man-Years: 2

Serial No. NCI-4014(c)
1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of the Immune Response in Normal and Pathological States

Previous Serial Number: SAME

Principal Investigator: Warren Strober, M.D.

Other Investigators: T.A. Waldmann, M.D. R.M. Gebhard, M.D. G.N. Rogentine, M.D., and D.L. Nelson, M.D.

Cooperating Units: Digestive and Hereditary Diseases Branch, NIAMDD

Man Years:

Total: 5
Professional: 3
Others: 0

Project Description:

Objectives: Studies were directed toward determining the nature and mechanism of circulating and cellular immune responses to antigenic challenges in animals and humans, including patients with immunodeficiency states such as gluten-sensitive enteropathy, dermatitis herpetiformis, ataxia telangiectasia, hypogammaglobulinemia, and intestinal lymphangiectasia. Insight into the cause of the high incidence of reticuloendothelial malignancies associated with these disorders was sought.

Methods Employed: A host of techniques were used to assess immunological states of patients. Antibody responses were assessed with hemagglutinin techniques as well as with a modification of the C¹a fixation and transfer test of Borsos and Rapp. Cellular immunity was assessed using lymphocyte transformation as well as skin test batteries. B-lymphocytes were quantitated with specific immunofluorescent antibodies and T-lymphocytes were quantitated using the sheep RBC rosette technique. In addition to these techniques, immunological function was studied with immunoelectrophoresis, radial immunodiffusion and metabolic turnover studies for measurement of immunoglobulin synthesis and catabolism.

Major Findings: To study the role of gluten sensitive enteropathy we have worked toward development of an in vitro model of this disease. We have taken advantage of the fact that jejunal specimens can be maintained

balance and Ca^{47} kinetic techniques with bone densitometry, we found that the drug decreases rates of turnover and prevents new ectopic calcifications from forming. In addition, the density of bone as well as growth and developmental parameters are unaltered by the drug. These findings support the early use of the drug in children with diseases of ectopic calcifications.

Calcium kinetic data from 10 patients with hypoparathyroidism were compared with data from 15 normals. Using compartmental analysis it was found that parathyroid hormone not only increases bone resorption but also increases the entry of calcium from rapidly turning over compartments into plasma. These findings suggest that parathyroid regulation of calcium transport processes may play a role in the hormone-dependent maintenance of plasma calcium homeostasis.

Significance to Biomedical Research: The maintenance of calcium homeostasis is vital to man. Small changes in plasma calcium ion activity profoundly disturbs many physiological processes. The effects are related to the interaction of calcium ion in maintaining cell membrane integrity. Activation of adenylyl cyclase, transport of substrates as well as energy metabolism may also be related to intracellular or extracellular calcium concentrations. In cancer patients with bony metastases hypercalcemia may occur because bone is a major tissue component in the calcium homeostatic system. Our studies using pharmacologic agents which affect calcification may elucidate the mechanism of calcification and provide a therapeutic approach in treating bone metastases, metabolic bone diseases, and disorders of calcium homeostasis. In addition, our studies defining the calcium absorption mechanism may provide a therapeutic approach in treating patients with calcium malabsorption states and metabolic bone disease.

Proposed Course: We are finishing our studies of the effects of diphosphonates on calcium and bone metabolism. With the completion of these studies, we are planning to terminate this project.

Honors and Awards

None

Publications

Phang, J.M. and Weiss, I.W.: The maintenance of calcium homeostasis in man. Invited chapter in Handbook of Physiology. G. Aurbach (ed.). In press.

Aurbach, G.D. and Phang, J.M.: Parathyroid hormone, calcitonin and vitamin D. Invited chapter in Medical Physiology. V. Mountcastle (ed.). In press.

in organ culture for 24-48 hours. During this time isolated tissue can be exposed to a variety of possible pathogenic influences and the effect of the latter on cellular function can be determined. Using an organ culture system, we first learned that jejunal tissue for the normal individuals undergoes and increase in the activity of intestinal enzymes, alkaline phosphatase, sucrose and trehalase during culture. This is most likely due to a maturational process of the epithelial cell which normally occurs in vivo. When jejunal specimens were obtained from patients with gluten sensitive enteropathy with active disease and placed into organ culture it was found that the alkaline phosphatase content of the biopsy tissue was initially low. However, during culture in the gluten free environment, activity rose to approach normal levels. This rise is significantly greater than that observed in normal control specimens when expressed as a fractional increase and, in addition, probably greater when expressed as an absolute increase. When compared with the in vitro behavior specimens obtained from patients with other GI diseases, the increase is clearly greater when expressed either way. Significantly, this change in enzyme activity was sometimes accompanied by ultrastructural changes in the epithelial cells: lengthening and increased number of microvilli. This rise in enzyme activity as well as the accompanying morphologic changes appear to be due to maturation of epithelial cells not possible in the gluten containing in vivo environment from which the tissue was taken.

In contrast with the behavior of biopsy material cultured in the absence of gluten proteins, specimens cultured in the presence of gluten proteins show no significant increase in epithelial cell alkaline phosphatase activity. This inhibitory effect of gluten protein is specific in that it is not seen in the culture systems obtained from normal or disease control patients; furthermore, another food protein, casein, does not have similar effects. Thus, organ culture of biopsy specimens from patients with gluten-sensitive enteropathy in the presence of gluten protein, represents an in vitro model of gluten-sensitive enteropathy.

Using this model we studied the effect of gluten proteins on the epithelial cells in vitro of specimens taken from patients with gluten-sensitive enteropathy in remission and the same patients following gluten challenge, that is, gluten-sensitive enteropathy in exacerbation. We found that gluten proteins prevent alkaline phosphatase increase only in specimens taken from patients with gluten-sensitive enteropathy in relapse and not in specimens taken from patients with quiescent disease who had been on a gluten-free diet for prolonged periods. This clearly indicates that gluten protein is not directly toxic to epithelial cells of patients but must first activate endogenous effector mechanisms which causes toxicity. This idea is further strengthened by the observation that when biopsy specimens of patients with gluten-sensitive enteropathy in remission are cultured in the same culture dish as biopsy specimens of patients with gluten-sensitive enteropathy in exacerbation, the remission specimens demonstrate gluten toxicity, i.e., they do not undergo increased alkaline phosphatase content. When the very same

specimens that are cultured alone they undergo an increase in alkaline phosphatase content regardless of the presence of gluten proteins. Presumably, the relapse specimens are in an activated state and release material which in turn can act on remission specimens in conjunction with gluten proteins.

In previous studies we have established that 90% of the patients with gluten sensitive enteropathy carry the HL-A histocompatibility type, whereas this type is found in only 20-25% of the random population. This relationship between HL-A8 and gluten sensitive enteropathy is one of the most striking yet found between a tissue type and a disease. The increased HL-A8 frequency in gluten-sensitive enteropathy is similarly increased in patients with dermatitis herpetiformis, a skin disease that is often associated with a GI lesion entirely similar to that found in gluten-sensitive enteropathy. In a study of the HL-A frequency in the dermatitis herpetiformis we found that HL-A occurred in some 60-65% of patients. This is the expected frequency based on the incidence of HL-A in the GSE population and the incidence of GSE lesion in patients with dermatitis herpetiformis. In recent studies we have also determined that severe gut lesions in dermatitis herpetiformis always occurs in these patients with HL-A8 whereas those without HL-A8 usually do not have the GI disease. Thus, these studies of dermatitis herpetiformis patients strengthen the association between histocompatibility type and the presence of gluten-sensitive gastrointestinal disease.

In other studies of patients with dermatitis herpetiformis we have established that patients with morphologic evidence of gastrointestinal disease, that is, the presence of villous atrophy, is very well correlated with increased IgA synthesis at local mucosal sites as determined with a solid phase immunoabsorbent system. The finding of this immunologic abnormality is parallel to previous studies in patients with gluten-sensitive enteropathy and suggests that an immunologic mechanism is important in the pathogenesis of dermatitis herpetiformis.

Studies of patients with intestinal lymphangiectasia have continued. Intestinal lymphangiectasia is a disease characterized by dilated intestinal lymphatics resulting in loss of lymph into the gastrointestinal tract. Patients with this condition have hypoproteinemia as well as lymphocytopenia and an accompanying immunologic defect. During the past year the effects of chronic lymphocytic loss into the gastrointestinal tract on the body lymphocyte economy has been studied. In particular, total B cell levels have been determined in a group of patients with this condition and it was determined that B-lymphocyte population. These findings indicate that B-lymphocytes are less subject to loss into the gastrointestinal tract than T-lymphocytes, a finding presaged by our previous studies of the functional capability of residual lymphocyte populations in this patient group.

Significance to Biomedical Research: Studies of patients with gluten-sensitive enteropathy have provided new insights into the operation of

the local mucosal immune system. They suggest that local responses may be under the control of immune response genes or, alternatively, they depend on the presence or absence of binding sites (receptors) which, in turn, controlled by a histocompatibility gene. If the latter possibility is true it may indicate that antigens entering the GI tract are more immunogenic if they become attached to the epithelial cell surface and thereby mimic antigens arrayed on particular organisms.

Studies of immune responses in patients with intestinal lymphangiectasia and other immune deficiency diseases are leading to a greater understanding of the site of molecular nature of defect in these various conditions. Patients with immune deficiency are more prone to develop neoplasms and is therefore probable that immunologic processes constitute an important defense against development of such neoplasms. Studies of the immune defects encountered in the immune deficiency states and other diseases involving immune functions will provide a greater understanding of how immune processes prevent the development of neoplasms.

Proposed Course: Continue studies of the role of immunologic factors in gluten-sensitive enteropathy and other gastrointestinal diseases will be carried out. In particular, the organ culture system will be utilized to show that antibodies with specificity for gluten protein are an essential component in the pathogenesis of gluten-sensitive enteropathy. This demonstration is now possible using the in vitro model of gluten-sensitive enteropathy that has been developed in the laboratory. It is proposed that patients with other gastrointestinal diseases will be studied with similar techniques. In particular, patients with inflammatory bowel disease will be evaluated using standard immunologic evaluation studies as well as studies of B- and T-cell populations.

Honors and Awards

None

Publications

Katz, S.I., Falchuk, Z.M., Dahl, M.V., Rogentine, G.N. and Strober, W.: HL-A8: A genetic link between dermatitis herpetiformis and gluten-sensitive enteropathy. J. Clin. Invest. 51: 2977-2980, 1972.

Falchuk, Z.M., Rogentine, G.N. and Strober, W.: Predominance of histocompatibility antigen HL-A8 in gluten-sensitive enteropathy. J. Clin. Invest. 51: 1602-1605, 1972.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-4014(c)

Title: Studies of the Immune Response in Normal and Pathological States

Principal Investigator: Warren Strober, M.D.

Section:

Lab/Branch: Metabolism

Goal: To study the nature and mechanism of the immunologic abnormality in patients with immunologic deficiency and patients with aberrations of the immune system.

Approach Patients with a wide variety of immunologic deficiency diseases including those with intestinal lymphangiectasia, ataxia telangiectasia hypogammaglobulinemia, and gluten-sensitive enteropathy (GSE) are studied with a broad range of tests of immunologic function. These include evaluation of responses to a standard antigens, and characterization of immunoglobulin levels and types. These also include tests of cellular immune function such as lymphocyte transformation and evaluation and quantitation of peripheral "T" cells. Patients with a broad range of gastrointestinal disease have been studied using short term in vitro culture techniques in association with solid phase immunoabsorbants. Patients with a gluten-sensitive enteropathy and other gastrointestinal diseases have been studied using in vitro organ culture techniques. The synthesis of immunoglobulin in vitro was studied with the culture of peripheral lymphoid tissue and evaluation of immunoglobulin synthesis in vitro using solid phase immunoabsorbants and radioimmuno-electrophoresis. Immunoglobulin synthesis in vivo was studied using metabolic turnover techniques employing radiolabeled purified immunoglobulins.

In the past year an in vitro model of gluten sensitive enteropathy has been developed. This model consists of the in vitro culture of

Progress: jejunal biopsy specimens of patients with GSE in the presence and absence of gluten proteins. Using this model we have determined that gluten proteins are not directly toxic to tissues of patients with GSE but must activate an endogenous effector mechanism of toxicity, presumably the immunologic system. In other studies we have determined that patients with GSE as well as those with a related disease, dermatitis herpetiformis, have a high frequency of the HL-A8 histocompatibility type. We have further shown that in dermatitis herpetiformis, HL-A8 is associated with a high incidence of GI disease while its absence is associated with a low incidence of GI disease.

Significance for Cancer Research (NCP Objective 2 Approach 1)
Patients with immunologic deficiency as well as those with immunologic observations have a high incidence of neoplasia. Through the study of such patients, the normal defenses against the development of cancer will be better understood.

Level of Effort: Professional Man-Years: 3

Serial No. NCI-4015(c)

1. Office of the Clinical Director
2. Metabolism Branch
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Studies of the Development and Function of Humoral and Cellular Immune Mechanisms of Host Defense

Previous Serial Number: SAME

Principal Investigator: R. Michael Blaese, M.D.

Other Investigators: Thomas Waldmann, M.D., Warren Strober, M.D., J.J. Oppenheim, M.D., J. Wunderlich, M.D., L. Altman, M.D., H. Hofstrand, M.D., S. Rosenoff, M.D., R. Young, M.D., and H. Kirchner, M.D.

Cooperating Units: Immunology Branch, NCI, and Laboratory of Microbiology, Immunology Section, NIDR, and Medicine Branch, NCI.

Man Years:

Total: 2
Professional: 2
Other: 2

Project Description:

Objectives: The objectives of this study were to determine mechanisms important to the development and function of both humoral and cellular immune response in animals and man; to determine the nature of the deficiency in immune function characterizing such disease states as the Wiskott-Aldrich syndrome, intestinal lymphangiectasia and advanced malignancy; and, to develop approaches to prophylaxis and/or therapy of the infectious and neoplastic disease frequently associated with defective immune processes. This work is directly related to Objective 2, Approach 1 of the National Cancer Plan.

Methods Employed: Antibody responses of experimental animals and man were determined after immunization with bacterial vaccines such as brucella abortus, *S. typhosa*, and pertussis; with particulate cellular antigens such as sheep, burro, and chicken RBC's; with purified protein antigens such as KLH, diphtheria and tetanus toxoids, guinea pig- γ -globulin and bovine γ -globulin; with purified polysaccharide antigens such as pneumococcal polysaccharides types I, II, III, and the Vi antigen of *E. coli*. The antibodies were detected by standard techniques or by newly developed micro methods of bacterial agglutination, red cell hemolysis, and passive hemagglutination using CrCl₃ as an agent to

couple the antigens to a carrier RBC surface. In addition, modifications of the Farr antigen binding assay were used.

Cellular immune responses were measured by delayed hypersensitivity skin tests, contact sensitivity to DNCB, and skin allografts. Lymphocyte transformation in vitro was carried out utilizing non-specific mitogens such as PHA, PWM and staph filtrate; specific antigens such as SLO, SKSD, diphtheria and tetanus toxoids, KLH and candida albicans; and allogeneic cells in mixed leukocyte culture. Lymphocyte function in vitro was also measured by production of mediators of cellular immunity such as lymphotoxin and MIF, and by cytotoxicity reactions with antibody sensitized target cells or with non-specifically mitogen activated effector lymphocytes.

Studies of the characteristics of various cell surface receptors on immunocompetent lymphocytes and monocytes-macrophages utilized autoradiography, immunofluorescence microscopy, and cellular rosette formation.

Major Findings: A broad study of immune function in patients with a variety of malignant and/or immunodeficiency disorders was continued. Principal among the diseases studied was the Wiskott-Aldrich syndrome an x-linked immune deficiency disease associated with a 33% incidence of malignancy in our series. Children with this syndrome were found to have profound defects in both humoral and cellular immunity characterized by clinical anergy and poor specific antibody production. Quite unlike the other congenital immunodeficiency disorders, however, is the finding that the effectors of immunity in these patients are generally intact. Thus, they were found to have normal numbers of circulating "T" and "B" lymphocytes as detected by microcytotoxicity or immunofluorescence assays with specific antisera. They produce immunoglobulin at normal or elevated rates. Their lymphocytes have normal proliferative responses in vitro to non-specific mitogens such as PHA and concanavalin A, and these stimulated cells produce normal amounts of various lymphokines including macrophage migration inhibition factor (MIF) lymphotoxin, and a chemotactic factor for monocytes (LDCF). In addition, the capacity of their lymphocytes to kill foreign cells is intact. Thus, their "B" lymphocytes kill target cells coated with appropriate antibodies called lymphocyte dependent antibodies (LDA), and their "T" lymphocytes will kill normally when activated by non-specific mitogens such as PHA. However, in spite of these normal non-specific in vitro tests of immunocompetence these patients lack adequate specific humoral or cellular immune responses both in vivo and in vitro. Thus, we believe that the immune deficiency in these patients is the result of a disorder in the afferent arc of immunity. The intact immune effectors are not being appropriately turned on; perhaps because of a defect in antigen recognition or processing. Our finding that several of these patients have transient immunoglobulin paraproteins and the occurrence of lymphoid malignancy in a high proportion of patients suggests that the normal regulatory mechanisms which control proliferation of immuno-

competent cells are also affected in this syndrome, probably as another manifestation of the same basic defect resulting in the immunodeficiency.

Many of the same tests of immune function described in the study of the Wiskott-Aldrich syndrome have been applied to other immune deficiency and malignant disorders. Lymphocytes from patients with CLL were found to be markedly defective in vitro in proliferation (blast transformation) target cell killing by both "T" and "B" cells, and lymphokine secretion following stimulation with both non-specific mitogens and specific antigens. The abnormal cell in the circulation of patients with CLL is lymphocyte derived from the thymic independent "B" cell series. When "T" lymphocytes were specifically purified from the blood of these patients, normal in vitro blast transformation, killer activity and lymphokine secretion could be demonstrated. Thus, dilution of normally responsive "T" lymphocytes by the defective malignant "B" cells in CLL is the cause of the immune deficiency of this disease.

In collaboration with Dr. Leonard Altman of the NIDR we have studied two additional mechanisms of defense against infection which may be abnormal in patients with immune deficiency or malignancy. As a result of the interaction of sensitized lymphocytes with antigen (micro-organism of tumor cell), the lymphocytes are stimulated to produce a variety of soluble biologically active substances called lymphokines. Some of our patients with immune deficiency or neoplastic disease are unable to produce some of these lymphokines, particularly a chemotactic factor for monocytes and macrophages (LDCF). This defect has been observed in a patient with chronic mucocutaneous candidiasis, in 4 patients with CLL, and in 4 patients with lymphoma. We have also described a previously unrecognized defect in host resistance in patients with chronic infection or malignancy. Anergic patients with chronic mucocutaneous candidiasis and Hodgkin's disease were studied who were unable to produce a normal inflammatory reaction because of failure of their monocytes and macrophages to migrate normally in response to this chemotactic lymphokine. Thus, even though some of these patients had normal lymphocyte responses to antigens, they could not produce effective immunity because the inflammatory cell response essential for antigen (micro-organism or tumor cell) destruction and removal was defective.

A particularly intriguing observation on one of these patients was that the defective monocyte responsiveness was corrected after the patient was treated with transfer factor. Extensive further investigations of the effect with transfer factor therapy on both specific and non-specific resistance are now underway in patients with Hodgkin's disease, non-Hodgkin's lymphoma, leukemia, ovarian carcinoma and a variety of immunodeficiency states.

Studies of various factors influencing the development of immunocompetence in the neonatal period were continued. We have previously shown that neonatal rats acquire immune responsiveness to different antigens in a distinct sequence rather than simultaneous in early post-natal life.

This apparent sequential maturation of immune competence was found not to be dependent upon stepwise differentiation of specific antigen reactive cells, but rather upon the maturation of components of the afferent limb of immunity. Macrophages play an essential role in the initiation of immune responses to many antigens, and it was demonstrated that immaturity of this system of cells accounted for much of the apparent immune deficiency in the neonate. Thus, when mature adult macrophages were given to the neonate, they produced antibody to a variety of antigens to which they were ordinarily unresponsive. It was also found that a variety of materials given to the neonates would result in accelerated maturation of their own macrophage population, and result in earlier immunocompetence. Thus, the adjuvant properties of such agents as endotoxin, BCG, alum, pertussis vaccine, silica, and poly A.U. were shown to function by macrophage activation. We have also shown that activation of the macrophage system of the neonate not only allowed antibody formation in otherwise unresponsive neonates, but also was effective in preventing the induction of specific immunological tolerance by high doses of antigen given at birth. This finding was utilized to prevent tolerance to the vertically transmitted mammary tumor virus in C₃H mice and this non-specific immunoprophylaxis subsequently resulted in resistance of these mice to mammary tumor transplantation. Macrophage activation in neonatal C₅₇ BL/6 mice, however, did not affect subsequent development of progressive tumors caused by neonatal infection with M-MSV.

Further studies of role of afferent limb were carried out to determine its effect on the cellular immune system. Adult macrophages and macrophage activating substances were found to enhance the ability of spleen cells from neonatal rats to induce a graft versus host reaction in F₁ hybrid recipients. Macrophage activation also promoted both the development of delayed type hypersensitivity and its expression by skin testing. Macrophage activation also significantly enhanced expression of cellular immunity to RNA virus associated tumor antigens in mice.

Investigation of the contribution of various lymphoid cell populations and the central lymphoid organs to immune responses in the chicken were continued. The chicken provides a unique experimental model for such studies because total ablation of the "B" cell-humoral immune system can be achieved in this species by surgical removal of the bursa of Fabricius shortly after hatching. Utilizing these "B" lymphocyte deficient agammaglobulinemic chickens, we have formally proven that such in vitro correlates of cellular immunity as blast transformation to mitogens like PHA, cellular lymphocyte killer activity, and lymphokine production are all properties of the "T" lymphocyte population. However, an unusual dissociation from the expected result was found in that in vitro lymphocyte blast transformation in response to specific antigens in the chicken appears to be a property of the "B" lymphocyte population.

Significance to Biomedical Research: The present studies extend our understanding of the diverse, yet interrelated mechanisms contributing

to the development and normal functioning of immune system in animals and man. They indicate that disorders of immune function may be related to the absence or malfunction of such central lymphoid organs as the thymus or bursa of Fabricius or to immaturity of such auxillary systems as the macrophage required for the processing of antigens in the initiation of an immune response. They also indicate that the macrophage plays multiple roles in a normal immune response, being both important in the initiation or afferent limb of such responses and also as the ultimate effector cell in many immune reactions. These observations on macrophage activation may provide an explanation for the mechanism by which such agents as BCG have shown some therapeutic effectiveness in the treatment of cancer. Our studies of lymphocyte function in patients with immune deficiency and advanced malignancy have extended our understanding of the nature of the reactions produced by different classes of lymphocytes and the observation that some of these functions may remain intact in severely anergic individuals may provide us with a mechanism of providing effective immunotherapy for malignancy occurring even in severely immune deficient patients. Similarly, the observation that the monocytes and macrophages are abnormal in some patients with cancer or immunodeficiency and that this defect may be corrected by transfer factor treatment offers another approach to utilize immune processes in therapy of disease.

Proposed Course: Continuing studies directed toward understanding the normal pathways involved in the development and function of the immune system and application of this new knowledge in approaches to the prevention and therapy of malignant disease.

Honors and Awards

None

Publications

Blaese, R.M., Oppenheim, J.J., Seeger, R.C., and Waldmann, T.A.: Lymphocyte-macrophage interaction in antigen-induced in vitro lymphocyte transformation in patients with the Wiskott-Aldrich syndrome and other diseases with anergy. Cell. Immunity. 4: 228-242, 1972.

Blaese, R.M., Rosenberg, E. and Wunderlich, J.: Evidence of two functionally distinct lymphocyte populations in man. In Jankovic, B.D. and Isakovic, K. (Eds.): Microenvironmental Aspects of Immunity. New York, Plenum Press, 1973, pp. 315-320.

Kirchner, H., Oppenheim, J.J., Blaese, R.M., and Hofstrand, H.J.: Defective in vitro spleen cell proliferative response to antigens in agammaglobulinemic chickens. J. Immunol. 109: 348-352, 1972.

Oppenheim, J.J., Kirchner, H., Jacobs, R.J., and Blaese, R.M.: Contribution of B and T lymphocytes to in vitro chicken proliferative spleen cell reactions to antigens. In Jankovic, B.D. and Isakovic, K. (Eds.): Microenvironmental Aspects of Immunity. New York, Plenum Press, 1973, pp. 141-148.

Strober, W., Blaese, R.M. and Waldmann, T.A.: Immunologic deficiency disease. Bulletin on the Rheumatic Diseases. 22: 686-691, 1972.

Waldmann, T.A., Strober, W. and Blaese, R.M.: Disorders of immunoglobulin catabolism. In Peeters, H. (Ed.): Protides of the Biological Fluids. Proceedings of the 19th Colloquium, 1971, Brugges, Belgium, Pergamon Press, 1972, pp. 425-428.

Waldmann, T.A., Strober, W. and Blaese, R.M.: Immunodeficiency disease and malignancy. Ann. Int. Med. 77: 605-628, 1972.

Weiden, P.L. and Blaese, R.M.: Hereditary thrombocytopenia: Relation of the Wiskott-Aldrich syndrome with special reference to splenectomy. J. Pediatrics. 80: 226-234, 1972.

Blaese, R.M., Weiden, P., Oppenheim, J.J., and Waldmann, T.A.: Phytohemagglutinin as a skin test for the evaluation of cellular immune competence in man. J. Lab. Clin. Med. 81: 538-548, 1973.

Blaese, R.M., Strober, W. and Waldmann, T.A.: Immunodeficiency in the Wiskott-Aldrich syndrome. In Bergsma, D. and Finstead, J. (Eds.): Proceedings of the Second International Workshop on Immunodeficiency Disease. In press.

Kirchner, H., Oppenheim, J.J. and Blaese, R.M.: The contribution of B cells to the in vitro proliferative reaction of chicken lymphocytes to non-specific mitogens and antigens. In Daguiard, F. (Ed.): Proceedings of the 7th Leukocyte Culture Conference. New York, Academic Press. In press.

Oppenheim, J.J., Blaese, R.M., Horton, J.E., Thor, D.E., and Granger, G. A.: Production of macrophage migration inhibitory factor and lymphotoxin by leukocytes from normal and Wiskott-Aldrich syndrome patients. Cell. Immunol. In press.

Sherwood, G. and Blaese, R.M.: Phytohemagglutinin induced cytotoxic effector lymphocyte function in patients with the Wiskott-Aldrich syndrome. Clin. Exp. Immunol. In press.

Snyderman, L., Altman, A., Frenkel, A., and Blaese, R.M.: Defective mononuclear leukocyte chemotaxis: A previously unrecognized immune dysfunction. Ann. Int. Med. In press.

Waldmann, T.A., Strober, W., Blaese, R.M., and Terry, W.: Immunoglobulin metabolism in disease. In Bergsma, D. and Finstead, J. (Eds.): Proceedings of the Second International Workshop of Immunodeficiency Diseases. In press.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-4015(c)

Title: Studies of the Development and Function of Humoral and Cellular Immune Mechanisms of Host Defense.

Principal Investigator: R. Michael Blaese, M.D.

Section:

Lab/Branch: Metabolism Branch, NCI

Goal: To gain insights into the basic mechanisms of resistance to neoplastic and infectious diseases by the study of experimental animal models and patients with immunodeficiency and/or malignant disease.

Approach General and specific aspects of immune function in patients with cancer and/or various immunodeficiency diseases are evaluated and experimental animal model systems are employed to define more completely the basic immune mechanisms suggested by these clinical observations. Humoral immunity is assessed by functional tests such as active antibody responses to various classes of specific antigens and determination of the cells participating in these reactions by immunofluorescence, autoradiography and microcytotoxicity assays. Cellular immune function is assessed by standard clinical measures as well as a wide range of in vitro tests including lymphocyte blast transformation, determination of lymphocyte "killer" activity in microcytotoxicity assays, and measurement of various lymphokines produced by activated lymphocytes. For example, clinical studies suggested a defect in immunity may arise from faulty recognition or processing of antigens. Cell transfer studies in rats utilizing many of the above listed techniques then defined the essential role of the macrophage in initiating specific humoral and cellular immune responses and defined a physiologic period in life in which immune deficiency is normally present because of immaturity of this accessory system of cells.

Progress: Extensive clinical studies have defined several immune defects in patients with Hodgkin's disease, CLL, chronic mucocutaneous candidiasis (CMC) and the Wiskott-Aldrich syndrome. Each had cellular immunodeficiency based on defective lymphocyte responses to specific antigens detected by lymphocyte transformation, lymphokine production, and lymphocyte killer activity. A previously unrecognized defect in the responsiveness of monocytes to lymphocyte produced chemotactic factor was found in patients with Hodgkin's disease and CMC. In one patient this defect, which may result in anergy even though immune lymphocytes function normally, was corrected by transfer factor therapy. Additional studies in neonatal rats and agammaglobulinemic chickens have demonstrated the role of the macrophage in the initiation of immune responses and the function of various specific lymphocyte subpopulation in cellular immune reactivity.

Significance for Cancer Research (NCP Objective 2 Approach 1)

The immune system plays an obvious vital role in defense against malignant disease as well as in controlling the infectious complications so common in these patients. The objective of this work is to gain further understanding of the immune process so that it can be more efficiently utilized in the therapy and prevention of cancer.

Level of Effort: Professional Man-Years: 2 years

SUMMARY STATEMENT
LABORATORY OF PATHOLOGY
DIVISION OF CANCER BIOLOGY AND DIAGNOSIS
NATIONAL CANCER INSTITUTE
July 1, 1972 to June 30, 1973

The diagnostic and research activities of the staff of the Laboratory of Pathology are listed by Sections. The research accomplishments are especially impressive when one considers that the majority of the staff participate to some extent in the diagnostic services for Clinical Center patients. In fact, because of changes in professional personnel, including resignations and retirement of some staff members, and because of additional administrative responsibilities of others, the overall diagnostic workload of several members of the staff has increased appreciably during the past year. It is by offering opportunities and facilities for laboratory research that we have been able to recruit and retain an outstanding group of anatomical pathologists who have been able to give vital support to the clinical programs of NCI as well as all the other Institutes with patients in the Clinical Center.

The research programs of the Laboratory have been involved with:

- A. Hematopathology and Immunopathology
- B. Viral Oncology and Cell Biology
- C. Comparative Pathology and Carcinogenesis
- D. Development and use of Automated Equipment for Cytology Diagnosis

Some of the more significant research accomplishments in which staff of the Laboratory participated, either as principal investigators or as collaborators include:

1. Studies of relationship of histopathologic types to prognosis in human malignant lymphomas.
2. Demonstration of chimerism in cell line of marmoset with Herpes-virus saimiri induced malignant lymphoma.
3. Studies of mechanism of action of interferon on viral messenger RNA in cell-free systems.
4. Demonstration of tubuloreticular structures in circulating lymphoid cells of patients with lupus erythematosus and induction of these structures in vitro by halogenated pyrimidines.
5. Detailed studies of extent of cytomegalovirus infections in children with leukemia.
6. Pathologic studies of graft versus host reaction in patients with bone marrow transplants.
7. Demonstration of mixed infection with arboviruses and oncornaviruses.

8. Histopathologic studies of American patients with Burkitt's tumors related to viral serologic data.

9. Demonstration of production of human chorionic gonadotropin in vitro by cell line derived from a human lung cancer.

10. Demonstration of histamine decarboxylase in malignant carcinoids of the Mastomys.

11. Induction of epidermoid carcinoma in rats by cigarette smoke condensate.

12. Induction of malignant mesotheliomas in rats by asbestos and fibrous glass with emphasis on role of fiber size.

Many members of the staff, in addition to their laboratory and service work, serve on numerous national and international committees, editorial boards, etc. Some of the principal activities of this sort are: Dr. Stanton, Scientific Editor of the JNCI; Dr. Dawe, Associate Editor for International Journal of Cancer, and Cancer Research; Dr. Rabson, Associate Editor of JNCI; Dr. Friedman, Associate Editor of Journal of Virology; Dr. Berard, Pathology Editor, Journal of the Reticuloendothelial Society; Dr. O'Connor, Chairman of the USA National Committee for UICC and Chairman of Commission on Epidemiology, UICC; Dr. O'Connor and Dr. Thomas, WHO Collaborating Centers for Classifications of Neoplasms; Dr. Dawe, Chairman of Committee on Comparative Oncology, UICC. Other committees, symposia, etc., participated in by the staff are listed in the annual reports.

Dr. O'Connor, in addition to the activities listed above, is currently assigned to the Office of Director, NCI as a Special Assistant to develop plans and programs for International Affairs for the NCI. During his absence of several months on this assignment, Dr. Powell has responsibility for the Surgical Pathology Section. The diagnostic services are shared by other members of the Laboratory of Pathology staff.

Dr. Gerhard Krueger resigned from the Hematopathology Section in June 1972 to return to Germany and accept a position as Head of Immunopathology, Department of Pathology, University of Cologne.

Dr. Philip Grimley resigned from his position as Head of the Ultrastructural Pathology Section in the Spring of 1973 to accept a position as Director of Clinical Pathology Laboratories and associate to the Director of the Clinical Laboratory Center, Division of Laboratories and Research, New York State Department of Health, Albany, New York.

Dr. Zsuzsa Schaff completed her assignment as Visiting Associate March 30, 1973 and returned to the Department of Pathology, Semmelweis Medical University, Budapest.

Dr. Costan Berard spent three months in Hiroshima, Japan, as a Visiting Scientist. During this time he was supported by the Japan Society for the Promotion of Science and worked both at Hiroshima University and at the Atomic Bomb Casualty Commission.

Dr. Gerald Berry spent two months in the fall of 1972 as a guest worker in Hematopathology during Dr. Berard's absence.

Dr. Stephen Doty is an NIH Fellow since September 1972 and is working with Dr. Banfield in electron microscopy and with the electron probe equipment on problems related to the identification of elements in tissues and cells.

Dr. Pelayo Correa, who is a Visiting Scientist with Mr. Haenszel in the Biometry Branch, NCI has continued working part-time in the Laboratory of Pathology. He has reviewed some animal material from chemical carcinogenesis projects and collaborates with Dr. O'Connor on several epidemiological studies, particularly of Hodgkin's disease.

Dr. Tsumuo Yamamoto started working as a guest worker in Hematopathology under Dr. Berard in April 1973. His assignment is for six months and he is supported by the Japan NIH and the ABCC where he is a staff pathologist.

Dr. Nubia Munoz is a Visiting Associate who started working in Dr. Rabson's laboratory in October 1972. She is on leave from the International Agency for Cancer Research in Lyon, France.

Dr. Frank Bastian worked as a guest worker in Dr. Rabson's laboratory from January to August 1972. He was supported by the Department of Pathology at Duke University.

During the summer of 1972, two Fogarty Scholars worked in the Laboratory of Pathology. Dr. George Klein from Stockholm worked with Dr. Rabson and Dr. Leo Sachs of Israel worked with Dr. Grimley.

The staff of the Laboratory of Pathology were either senior authors or co-authors of 62 papers and abstracts published in 1972. There are 10 publications to April 1973. (See attached list of publications).

Surgical Pathology Section:

Dr. O'Connor and Dr. Powell, together with the residents and other pathologists participating in the diagnostic services of the Laboratory of Pathology, are responsible for the diagnostic reports in Surgical Pathology. Approximately 6462 specimens were accessioned. These included approximately 2800 bone marrows sectioned and processed in the laboratory. Dr. O'Connor and his staff regularly make rounds with Dr. Ketcham and his staff in the Surgery Branch of NCI and show most of the surgical biopsies and operative specimens at the weekly Surgical Pathology Conference. Other conferences based on surgical or biopsy material are conducted with clinical groups including the hematology-lymphoma conference (Dr. Berard); the lymphoma staging conference (Drs. Thomas and Berard); the liver conference (Dr. Jaffe and Dr. O'Connor); and the radiation therapy conference (Dr. Thomas).

Postmortem Section:

Dr. Thomas, the residents and other staff were responsible for 224 autopsy examinations. A gross review of organs is held with the residents and clinical staff each morning at 8:30 a.m. and the staff and residents present

selected autopsies at numerous clinico-pathologic conferences. A new autopsy form first used in 1972 allows enucleation of eyes as part of the postmortem examination. Approximately 140 pairs of eyes have been removed and examined by the pathology staff of the National Eye Institute during the first year of this new program. A review of this program recently revealed that permission to remove eyes has not decreased the overall rate of autopsy permission given for patients who die in the Clinical Center.

Publications of studies on human material:

A number of studies on human disease were published by the residents and staff during 1972 and other studies are now going on which will lead to publication. These include Hodgkin's disease and other malignant lymphomas (Thomas 2, 24, Berard 22, 23, Berard and Thomas 24); medullary carcinoma of thyroid gland (Grimley 4); liver cell carcinoma in Ugandans (O'Connor and Traika 5); dinitrochlorobenzene sensitization (Rabson 8); congenital and acquired heart disease (Buja 14); bone marrow transplantation (Berard 25, 31); cancer at site of prior benign lesion (Dunham 26); lung cancer in U.S. veterans (Herrold 34); Herpesvirus hominis in trigeminal ganglion (Rabson 38); Burkitt's tumor (O'Connor and Berard 43, O'Connor 53, Krueger and O'Connor 56); tubuloreticular structures (Grimley 44); cytomegalovirus infections (Henson 50); acute tubular necrosis (Jaffe 51); metastatic melanoma of gall bladder (Jaffe 52); detection of alpha-fetoprotein in liver cancer (O'Connor 54); tissue culture of human breast carcinoma (Chu 57). Reference numbers are to 1972 publications list.

Other research activities by staff in Surgical Pathology and Postmortem Sections:

In addition to his diagnostic pathology services and administrative work, Dr. O'Connor conducts research in electron microscopy and in epidemiology. Included are published studies on antibodies to EBV in Burkitt's tumor patients, and on the epidemiology of Burkitt's tumor and Hodgkin's disease. His review of tumors of the respiratory tract will appear in Dr. Templeton's book on Cancer in Uganda. In collaboration with Dr. Krueger he has published studies of the ultrastructure of thymic lymphomas produced by persistent antigenic stimulation and immunosuppression.

Dr. Powell, in addition to his diagnostic service responsibilities which have greatly increased this past year, is collaborating on a number of studies of human diseases. In press are articles on morphology of the ovaries in women with anosmia and hypogonadotrophic hypogonadism, tumor immunotherapy with BCG, diffuse amyloidoses of the tracheobronchial tree, and Pneumocystis carinii pneumonia in cancer patients. Experimental work includes studies on testicular morphology in zinc deficiency and delay of sexual maturation in bulbectomized rats.

A complete list of other human and experimental studies by the staff and residents is given in the individual annual reports.

Laboratory services for human tissues:

Miss Bowling and her staff continued to provide excellent histological slides and special stains for human tissues. She also provided valuable consultation

on special staining and preparation of human tissues to many clinical associates and other NIH scientists. During the year she developed a rapid staining method for Pneumocystis carinii organism and a paper on this staining method is in press. She is currently preparing a 2nd Edition of the Histopathology Laboratories Procedures Manual which was first published in 1967.

Photography services for human tissues was provided by Mr. Edwards and Mr. Banks, who, in addition maintains the slide files which contain more than a million slides. Access to these and to the gross and microscopic photographs is invaluable for patient diagnosis and follow-up and for an increasing number of clinico-pathologic studies.

During the year, Miss Beaston was assigned to the pathology data processing unit and worked with Dr. Henson and Dr. Thomas on the storage, computer-based encoding and retrieval of pathology diagnoses. This system which was developed over a number of years in collaboration with Dr. Arnold W. Pratt, Director of DCRT is now capable of automatic, computer encoding of more than 80% of all pathology diagnostic statements. Unfortunately this data processing activity will be seriously affected by the recent resignation of Miss Beaston.

Cytopathology Section:

Dr. Chu and the staff are responsible for all diagnostic reports on exfoliative cytology specimens which include sputum, urine, cervical-vaginal smears, CNS fluid, effusions, etc. In addition, she and her staff are responsible for examining cellular materials for karyotyping and chromosome abnormalities. Approximately 4422 accessions (9487 smears) and approximately 171 chromosomal analysis specimens (1710 smears) were processed and examined in 1972. In collaboration with the staff of the Surgery Branch, Dr. Chu evaluated the reliability of cytologic diagnosis of fine-needle aspiration of enlarged nodes and other nodules in patients clinically diagnosed as having metastatic cancer. Results of these studies were given at a work-shop at the meeting of the American Society of Cytology.

Studies published during the year include urinary cytology as an indication of bladder neoplasia in mice with Dr. Malmgren and Mr. DelVecchio, chimerism in a lymphoid cell culture line derived from a Herpesvirus saimiri induced lymphoma in marmosets (Chu and Rabson 6) and cytologic studies of cultured cells from human breast cancer.

Automation of Cytology Diagnoses: Since July of 1972, Dr. Herman, a resident in the Laboratory of Pathology, has been investigating the capabilities of the Coulter Counter Model B and the Cytofluorograph to sense cell volume, surface area and two wavelengths of fluorescence of dispersed cells in a continuous flow system. This work, which initially used model systems (latex particles, pollens, red blood cells, and pure epithelial cell suspensions), now involves use of human gynecologic cytology specimens. Dr. Herman is also Project Officer for a contract which involves the development of a multi-parameter cell-sorter at the Los Alamos Scientific Laboratory. This equipment will be used for physically separating definable populations of abnormal cells from normal cells in human cytological material. The current work with the Coulter Counter and Cytofluorograph is directed at problems of specimen

collection and preparation so that sensed parameter ranges of normal and abnormal cells can be established. This scientific effort involves complete cooperation between Dr. Herman and his technician and the staff in the Cytopathology Section. Work on this project by the Cytopathology technical staff will increase in the next year after the cell-sorter being built at LASL is delivered to the Laboratory of Pathology. More details about this important program in automation of cytodiagnosis are in Dr. Herman's report and in the report of the meetings of the Committee on Cytology Automation.

Ultrastructural Pathology Section:

In addition to participation in the diagnostic services of the Laboratory of Pathology, Dr. Grimley and Dr. Henson carried out a large number of experimental research projects.

Dr. Grimley reports that a number of research projects initiated in previous years were completed in FY 1973. A study of the anti-viral action of several rifampicin derivatives led to the finding of an amino-substituted derivative with enhanced activity (45). Studies of specific membranous structures infected by the Group A arboviruses demonstrated a membrane-associated replication complex (19, 37). In mouse cell cultures, simultaneous replication of Group A arboviruses and C-particles was demonstrated and the possibility of a relationship to enhancement of murine leukemia by arbovirus infection was investigated (JNCI, in press). An effect of virus transformation on alkaline phosphatase in human thyroid medullary carcinoma was reported (4). A prospective clinical study of tubuloreticular structures (TRS) in lymphocytes of patients with LE and other connective tissue diseases was completed and published (44). Also, Dr. Grimley, as co-author with Dr. George Glenner completed a fascicle on Tumors of the Extra-Adrenal Parganglion System which will be published as one of the series of atlases by the AFIP.

Dr. Grimley plans to resign from the Laboratory of Pathology toward the end of FY 1973 to accept a position as Director of Clinical Pathology Laboratories in the Division of Laboratories and Research, New York State Department of Health, Albany, New York.

Dr. Henson completed and published studies on mouse cytomegalovirus infections (16, 30, 33) and human cytomegalovirus infection in leukemic patients (50). An equine herpesvirus infection of the central nervous system in rabbits (12) was also published in 1972. His main research activities now involve the preparation and purification of specific antibody and labeling reagents to study the membrane surface distribution of a variety of proteins and protein binding sites.

Hematopathology Section:

Dr. Berard has major responsibilities for diagnostic services for patients with diseases of the hematopoietic and lymphoreticular tissues. These include materials from patients in the Clinical Center and also submitted materials (slides and tissues) from patients included in the cooperative clinical studies by the Eastern Cooperative Oncology Group. In addition, he received slides or tissues on 116 cases of suspected malignant lymphoma which

were sent to him for consultation by pathologists in the United States and foreign countries. He was author or co-author of a number of studies on human diseases published during the year (see citations in earlier section of report).

Dr. Berard and Dr. Jaffe have developed a major research program in collaboration with Dr. Ira Green and Dr. Ethan Shevack (NIAID) to study surface membrane markers of reticuloendothelial cells (T and B lymphocytes and macrophages) in tissue sections of reactive and neoplastic diseases in humans. These studies have enormous implications for yielding information of both etiologic and therapeutic significance within the field of neoplastic hematology. Dr. Braylan who will join his staff as a Senior Staff Fellow in July will expand these studies to include transmission and scanning electron microscopy.

Dr. Berard spent three months (October-December) as a Visiting Professor in Japan sponsored by the Japan Society for the Promotion of Science. During this period he reviewed over 400 cases of reticuloendothelial disease at Hiroshima University and in the files of the Atomic Bomb Casualty Commission.

Dr. Yamamoto from the ABCC started working as a guest worker in Dr. Berard's section in April 1973.

Viral Oncology and Molecular Pathology Section:

Dr. Rabson, Dr. Friedman and Dr. Levin have continued their studies of oncogenic viruses and mechanisms of interferon action. Sixteen publications from these studies are listed in the bibliography.

Dr. Friedman has been at the National Institute for Medical Research in London during the year working with the biochemistry group on mechanisms of action of interferon in cell-free protein synthesizing systems. They have developed a cell-free system which translates viral RNA and have shown the effect of interferon on production of viral peptides. They are now continuing studies on the molecular mechanism by which interferon inhibits translation of the viral RNA.

Dr. Levin has been studying mixed infection of murine cells with arboviruses and oncornaviruses. She has shown that arbovirus replication is not affected by chronic productive infection with murine leukemia virus and that the budding and release of the murine leukemia virus continues throughout the replicative cycle of the arbovirus.

Dr. Rabson has continued his work with Herpesvirus saimiri. In collaboration with Dr. George Klein of the Karolinska Institute and Drs. Pearson and Ablashi of NCI, he has studied antibodies to early and late antigens of H. saimiri in normal and tumor-bearing animals. They have shown that tumor-bearing animals have antibody to both early and late antigens while sera of infected animals without tumor have antibody only to the late antigens. The similarities to EB virus infection in man are remarkable and suggest that H. saimiri in monkeys is an excellent model system. Dr. Rabson and a postdoctoral fellow, Dr. Bastian were the first to demonstrate that Herpesvirus hominis can be latent in the human trigeminal gland removed at autopsy and that the virus can be activated and recovered in culture. In collaboration with Drs. S. Rosen and B. Weintraub

of NIAMDD, Dr. Rabson has established cell line from a lung tumor of a Clinical Center patient and the cells in vitro produce human chorionic gonadotropin and placental alkaline phosphatase. It has also been shown that the cells produce an excess of the alpha subunit of hCG; a situation similar to that of plasma cell tumors which produce excess amounts of one of the immunoglobulin chains.

Comparative Oncology Section:

Drs. Dawe, Stanton, Snell, Banfield, Dunham and Mulay are the professional staff members of this Section. Dr. Doty is an NIH Fellow working with Dr. Banfield.

The experimental studies include: (1) morphologic characterization of neoplasms in a variety of laboratory animals including rodents, primates, lower vertebrates and invertebrates; (2) mechanisms involved in tumor development; (3) chemical and particulate fiber carcinogenesis; (4) cellular interactions between epithelium and mesenchyme; (5) study of cultured cell lines from a number of animal tumors; and (6) qualitative and quantitative analysis of elements using the scanning electron microscope and electron probe and ion probe. Publications included studies of the epithelial-mesenchyme interactions during polyoma virus-induced tumor formation of mouse salivary gland (Dawe 48, 60); on the role of fiber size in mesothelioma induction with asbestos and fibrous glass (Stanton 7); induction of epidermoid carcinoma in the lungs of rats by cigarette smoke condensate (Stanton 39); tumor of testes and seminal vesicles, and gastric carcinoids in the *Mastomys* (Snell 46); herpes-type virus in oysters (Banfield 41); and a review of benign lesions preceeding cancers of the skin and mucous membranes in man (Dunham 26).

Mr. Ralph Isenberg provided photographic services for the experimental studies and photomicrography for publications by the staff of the Laboratory of Pathology. He completed a consolidation of his photography laboratory with equipment from the photography laboratory formerly housed in Building 8 which was used by Dr. Grimley and Dr. O'Connor and their staff for EM photomicrographs.

Pathological Technology Section:

Miss Sanders and her staff processed and stained approximately 30,000 slides of animal tissues for members of the staff of the Laboratory and other scientists in NCI. During the year she and her staff successfully moved from Building 8 to Building 37 without appreciable interruption of the histopathology services. A technical manual "Animal Histology Procedures of the Pathological Technology Section of the NCI" was published by Miss Sanders in 1972 (DHEW Publ. No. (NIH) 72-275). Mrs. Coolidge published an article on the use of glycol-base antifreeze as a clarifier for frozen sections (27).

In addition, Miss Sanders spent several weeks as a consultant to help set up histopathology laboratories for the FDA (Arkansas) and for the Gallup Indian Medical Center (New Mexico). She also supervised the Baker contract for the cutting and staining of approximately 30,000 slides during 1972.

Serial No. NCI 500

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Histogenesis and Pathology of Induced and Spontaneous Tumors
of Laboratory Animals

Previous Serial Number: Same

Principal Investigator: Katharine C. Snell, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1972 - June 30, 1973)

Total:
Professional: 2
Other:

Project Description:

A. Praomys (Mastomys) natalensis: (Mastomys)

Objectives: (1) To study and describe the tumors and other lesions that develop spontaneously, some of which appear to be counterparts of human diseases. (2) To study the enterochromaffin and enterochromaffin-like cells of the gastrointestinal tract of normal Mastomys and of those that have primary gastric carcinoids.

Methods Employed: (1) Most Mastomys are allowed to live as long as possible to furnish information on tumor incidence. Prior to necropsy, blood serum from each animal is collected and frozen. This will be used for future determination of immunoglobulins in animals with glomerulonephritis, the relationship of autoantibodies to striated muscle in animals with thymoma, and the histamine content in animals with carcinoids of the glandular stomach.

(2) For collaborative experiments, Mastomys have been sent to Dr. Rolf Håkanson of Lund, Sweden and to Dr. Enrico Solcia of Pavia, Italy. Papers are in press describing their findings in the study of the argyrophilic gastric carcinoids.

Major Findings: (1) Malignant argyrophilic carcinoids occur in 1/2 or more of Mastomys 18 months of age or older. Thymomas and thymic hyperplasia, sometimes associated with myositis and myocarditis, appear in a high percentage of animals. When the Mastomys of Dr. Carel Hollander of Rijswijk,

The Netherlands, were combined with the NIH strain to make a total of 391 males over 18 months of age we have reported 9 adenocarcinomas of the rete testis, 6 seminomas, and 12 carcinosarcomas of the seminal vesicles.

(2) In a search for the parent cell of the gastric carcinoid of Mastomys, Dr. Håkanson has determined that there are two types of histamine-containing argyrophil endocrine-like cells in the oxyntic gland area and that a common progenitor of both types must represent the parent cell of the tumors. He reached this conclusion by the use of o-phthalaldehyde-induced fluorescence, silver staining and by electron microscopy. Dr. Solcia, using electron microscopy, is reporting that the probable progenitor of the gastric carcinoids is the enterochromaffin-like cell, but that the ultrastructural pattern of tumor cell granules differs from those of the normal ECL cells of Mastomys. Dr. Solcia also found a diffuse hyperplasia of endocrine cells displaying all staining and ultrastructural features of ECL cells.

Significance: Mastomys are becoming of increasing importance as experimental animals, since in many ways their diseases parallel those of man (gastric carcinoids, osteoarthritis, glomerulonephritis, multiple endocrine adenomas, for example). These animals may be useful for determination of the chronologic and functional relationships between thymoma and anti-striated muscle antibodies, and possibly polymyositis and myasthenia gravis. It seems likely that study of their gastric carcinoids may shed light upon the relationship between various types of enterochromaffin cells, pluriglandular adenomas and the production of histamine. The continued study of untreated Mastomys is important in order to establish a baseline for future experimental work.

Proposed Course: Collaborative studies will be continued. Old Mastomys now on hand will be necropsied and histologic examination of their tissues made. Hopefully a monograph on the tumors and other lesions will be written within the next year or so. Because of space restriction, we do not intend to continue the study of aging Mastomys beyond those animals now on hand.

B. Study of the Offspring of Pregnant Rats Treated with Diethylstilbesterol

Objectives: To see whether it is possible to induce tumors in the offspring of rats by treatment of the mothers when pregnant and thereby shed more light on the recent work of Herbst et al (New England J. Med. 284: No. 16, 1971) and of Greenwald et al (New England J. Med. 285: No. 7, 1971). These authors found that the rare tumor, adenocarcinoma of the vagina, was appearing in a number of young women whose mothers were treated with diethylstilbesterol during pregnancy for threatened abortion.

Methods Employed: Pregnant F344 and SD/N rats are being treated by the administration of diethylstilbesterol by stomach tube in doses equivalent for the rat to those given pregnant women. The drug is given on days 12 through 15 of pregnancy. We are putting aside the offspring of these rats for killing when they are about 1 year old in order to determine whether either the males or females develop any unusual tumors, particularly of the

genital tract.

Major Findings: No results as yet. Experiment has been hampered by difficulties in breeding both experimental and control rats. Diethylstilbesterol, given as late in pregnancy as day 12, does not increase the number of fetuses resorbed or aborted or the death of newborn animals over those not treated.

Significance: If tumors are induced in offspring of treated rats, it will help substantiate the inferred relationship in the human patients of prenatal diethylstilbesterol and the development of genital tract tumors.

Proposed Course: Continue the experiment.

C. Untreated Rats

Objectives: To determine the incidence of tumors and other lesions that occur spontaneously in the inbred Sprague-Dawley strain of rats. This is a relatively new strain developed by Dr. Carl Hansen, Laboratory Aids Branch. It is being used by several investigators but no pathologic study has been made on the animals.

Methods Employed: Weanling inbred Sprague-Dawley rats are being set aside for aging and groups of both males and females will be killed at 3-month intervals, beginning when the animals are 12 months old. The longevity of the rats will determine the oldest age group to be studied, but this will probably be from 27-30 months.

Major Findings: None so far.

Significance: A careful pathologic study will be useful to any investigator using these rats for experimental purposes because it is important to know the spontaneous incidence of tumors and other lesions. Also the knowledge gained from a study of neoplasms and other lesions that occur in laboratory animals can provide a basis and possible analogy to the study of the tumors and other lesions that occur in human beings.

Proposed Course: If space for aging large numbers of rats becomes available, we propose to study other recently developed inbred strains.

D. Monkeys

Objectives: Originally the objective of this experiment was to determine whether 3-methylcholanthrene injected into the wall of the stomach would induce gastric carcinoma. Since, after many years of observation, no gastric cancers have been found, we are keeping the remaining monkeys to study any tumors or other lesions that may occur with age.

Methods Employed: 3-methylcholanthrene was injected or implanted in the wall of the stomach of rhesus monkeys 18 to 19 years ago. The monkeys, which are now 23 or more years old are being observed and allowed to live as long as possible. Prior to necropsy, skeletal surveys will be made by Dr. Doppman of the Clinical Center X-ray department. Also, brains will be perfused by Dr. Henryk Wisniewski of the Albert Einstein College of Medicine, who is interested in studying the aging process of neurons by electron microscopy.

Major Findings: Nothing significant has been observed in the stomach of any monkey necropsied. Three cases of spinal ankylosis, bilateral carcinoma of the kidney, diabetes with hyalinization of the islets of Langerhans, and adenocarcinoma of the ascending colon are the principal pathologic findings so far observed.

Significance: With the increasing use of monkeys in cancer research it is important to study old animals to determine the incidence of spontaneous disease. Very few colonies of monkeys of known and advanced age are in existence so that our animals constitute an important group for future study. Hyperostotic spondylosis is a disorder that is currently the subject of considerable clinical study. These monkeys may offer an opportunity for observing changes in bone which are almost never available in human autopsy material. The finding of carcinomas of the kidney and of the colon as well as the hyalinized islets in the diabetic monkey suggest that monkeys, if allowed to live long enough, may indeed develop many of the same diseases found in man.

Proposed Course: Continued observation of monkeys with necropsies as necessary with collaborative studies as indicated under Methods.

E. Transplantation of Tumors

Objectives: To carry interesting or unusual tumors in transplant for possible future study in this laboratory or by other investigators.

Methods Employed and Major Findings: The following tumors are carried by subcutaneous, intraperitoneal, or intramuscular injection:

- 555..Gastric adenocarcinoma induced by 2,7-FAA in ACI/N rat. Tumor develops metaplastic bone, bone marrow and cartilage in center.
- 328..Gastric adenocarcinoma, C57BL mouse, induced by intramural injection of 3-MCA into the wall of the stomach. Mice in which this tumor has grown for a long time develop hypervolemia. Currently being studied is tissue culture by Dr. L.M. Edelstein of New England Medical Center, Boston.
- 586..Skin appendage tumor induced in ACI/N rat by oral administration of 2,7-FAA, being studied by Dr. Shu Wen How and in tissue culture by Dr. L.M. Edelstein of the New England Medical Center, Boston.

673,684,691..Malignant argyrophilic carcinoids that originated in the glandular stomach of untreated Mastomys.

599..Gastric adenocarcinoma induced by 2,7-FAA in ACI/N rat.

Publications:

1. Snell, K.C., and Hollander, C.F.: Tumors of the testes and seminal vesicles in Praomys (Mastomys) natalensis. J. Natl. Cancer Inst. 49: 1381-1393, 1972
2. Håkanson, R., Larsson, L.I., Owman, C., Snell, K.C., and Sundler, F.: Fluorescence and electron microscopic histochemistry of endocrine-like cells in gastric mucosa and argyrophil tumor of Praomys (Mastomys) natalensis. Z. Zellforsch. (in press)
3. Capella, C., Solcia, E., and Snell, K.C.: Ultrastructural study of the endocrine cells and argyrophil carcinoids of the stomach of Praomys (Mastomys) natalensis. J. Natl. Cancer Inst. (in press, to be published in June, 1973)

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-500

Title: Histogenesis and Pathology of Induced and Spontaneous Tumors of Laboratory Animals

Principal Investigator: Katharine C. Snell, M.D.

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To study the histogenesis and pathology of induced and spontaneous tumors and associated lesions and physiologic changes in laboratory animals so that the similarities to and differences from human tumors may be ascertained.

Approach: Untreated Mastomys are being allowed to live as long as possible in order to study the incidence of tumors and other lesions that occur with advancing age. In a cooperative project with Dr. E. Solcia (Italy) and Dr. R. Håkanson (Sweden), the carcinoids of the stomach are being examined by electron microscopy and histochemistry to determine their parent cell and its relationship to other endocrine cells of the gastrointestinal tract. Certain strains of inbred rats are being killed at intervals, mainly to ascertain what lesions the rats develop and whether these strains might be suitable experimental animals and for what experiments they might be useful. Monkeys over 20 years are being examined for similar reasons. Pregnant rats are treated with diethylstilbesterol in doses equivalent for the rat to those given pregnant women about 20 years ago and thought to induce carcinoma of the vagina in the offspring. The rat offspring are put aside. Some will be killed at maturity, and others at a later date, to see whether either the males or females develop any unusual tumors, other lesions, or early precancerous changes, particularly in the genital tract.

Progress: One half or more of old Mastomys develop argyrophilic gastric carcinoids, the progenitor of which may be either an enterochromaffin-like cell or a precursor of the 2 types of histamine-containing argyrophil cells of the gastric mucosa. Thymomas are sometimes associated with myositis and myocarditis and with autoantibodies to striated muscle. Glomerulonephritis and degenerative joint disease in Mastomys are similar to these diseases in man. The tumors of aged Rhesus monkeys include carcinoma of the kidney and adenocarcinoma of the colon. Diabetes and spinal ankylosis also occur. Not enough offspring of DES-treated rats have been examined to reach any conclusion.

Significance for Cancer Research (NCP Objective 3 Approach 1)

If experimental animals such as rats, Mastomys and monkeys are to be used as tools for the study of cancer in man, it is important to know exactly how the animal tumors and physiologic changes in the host resemble and differ from comparable tumors in man. The response, or lack of it, in the offspring of pregnant rats treated with diethylstilbesterol may help elucidate the problem of the relationship of hormones and the time of their administration to carcinogenesis.

Level of Effort: Professional Man-Years: 2

Serial No. NCI 501

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Carcinogenesis in the Cheek Pouch of Hamsters or in the Esophagus of Hamsters: A Search for Factors in the Environment that may be Related to Cancers in Man

Previous Serial Number: Same

Principal Investigator: Lucia J. Dunham, M.D.

Other Investigators: Roger W. O'Gara, M.D. (Deceased)

Man Years (July 1, 1972 - June 30, 1973)

Total: 3/4

Professional: 1/4

Other: 1/2

Project Description:

Objectives: To identify substances or factors in the human environment that induce or influence the development of cancers.

Methods Employed: 1) The effects of several plants used for herb teas (Annona, Heliotropum, Krameria, Acacia, under consideration as possible causes of cancers of the esophagus in Curacao, NWI) were tested in the hamster cheek pouch or esophagus with and without added injury to tissues by calcium hydroxide (lime).

2) The effects of arecoline, an alkaloid present in the betel quid used in Southeast Asia, was tested in hamsters by oral administration, and application to the cheek pouch.

3) The abdominal portion of the esophagus of hamsters was chronically constricted by means of surgically placed cuffs of polyethylene tubing, and carcinogenesis was attempted by means of intra-esophageal instillation of benzyrene.

4) Dunham served as a consultant in connection with the posthumous studies of plant materials conducted in the laboratory of Dr. O'Gara. She has prepared an article for publication with G. Kapadia, C. Lee, and J. Morton.

Major Findings: 1) In an earlier experiment, Annona muricata plant produced advanced atypia in a cheek pouch and an esophagus of hamsters, and Heliotropum ternatum apparently produced an esophageal papilloma. A current

experiment, nearly completed, involved intensive treatment of upper gastrointestinal sites with Annona and Heliotropum, and also with Krameria and Acacia which have produced sarcomas at subcutaneous sites in rats. In the new experiments Annona apparently produced a superficial spreading carcinoma in a hamster cheek pouch. Though Acacia brew administered to rats is strongly sarcomagenic (O'Gara: unpublished observations), highly concentrated Acacia brew administered to hamsters by mouth has so far not proved carcinogenic.

2) The extended experiments with arecoline have not resulted in the production of additional papillomas of esophagus, though this lesion was found in an earlier test of arecoline. Of 8 hamsters that had cheek pouches painted with arecoline in DMSO, 1 developed a squamous cell carcinoma of the forestomach.

3) An experimental group of hamsters with restricting cuffs at esophagus are under observation. Intra-esophageal instillation of benzpyrene in DMSO resulted in esophageal papillomas above the restricting cuffs in 2 animals.

Significance: The frequencies of cancer in the human mouth and esophagus vary widely by geographic area, and the ratios by sex vary also. Constrictions, dilatation, esophagitis, and scarring are among precursor lesions of cancer of the esophagus that have been reported. Environmental factors may be important in the genesis of this cancer, and also in cancer of the mouth which is suspected of being related to such chewing habits as betel quid, or nass, or tobacco. There is believed to be an urgent need for methods of assaying the carcinogenic effects of environmental factors.

Proposed Course: Groups of hamsters are currently fed powdered chow containing Annona muricata plant, or snuff, or arecoline, with and without added calcium hydroxide, to investigate their effects on the gastrointestinal tract. In another experiment, hamster cheek pouches are treated with a known carcinogen (DMBA) with and without added calcium hydroxide, to ascertain any effect there may be of the added alkali.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-501

Title: Carcinogenesis in the Cheek Pouch of Hamsters or in the Esophagus of Hamsters: A Search for Factors in the Environment that may be Related to Cancers in Man

Principal Investigator: Lucia J. Dunham, M.D.

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: A search for factors in the environment that may be related to cancers in man.

Approach: Studies of plants used for medicinal brews, and of related materials in the environment as they may affect the hamster cheek pouch or esophagus.

Progress: A few lesions that were produced at these sites in hamsters were histologically identical with carcinoma in situ or other proliferative lesions that sometimes precede cancer.

Significance for Cancer Research (NCP Objective 1 Approach 1)

Some plants that were tested are being investigated further as a possible cause of a cancer in man that may be unusually susceptible to changes in the environment, i.e. cancer of the esophagus.

Level of Effort: Professional Man-Years: 1/4

Serial No. NCI 501a

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Geographic Pathology Field Study on Uterine Cancer in Negro Women in New York City and the Washington, D.C. Metropolitan Area

Previous Serial Number: Same

Principal Investigators: Lucia J. Dunham, M.D., Louis B. Thomas, M.D.,
and Harold L. Stewart, M.D.

Other Investigators: None

Man Years (July 1, 1972 - June 30, 1973)

Total: 1/2

Professional: 1/2

Other: 0

The article was submitted to the Journal of the National Cancer Institute on March 8, 1972. It was returned to the authors with the suggestion that it be revised and resubmitted. A revision was prepared and is currently being critically appraised.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-501a

Title: Geographic Pathology Field Study on Uterine Cancer in Negro Women in New York City and the Washington, D.C. Metropolitan Area

Principal Investigator: Lucia J. Dunham, M.D.; L.B. Thomas; H.L. Stewart

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: Report of field study of uterine cancer in black women in New York City and in the Washington, D.C. metropolitan area.

Approach: Parallel study of incidence rates, histologic diagnoses, and factors obtained at interview of patients.

Progress: Study completed. A report prepared and recently submitted to JNCI was returned to the authors and is being revised.

Significance for Cancer Research (NCP Objective 5 Approach 1)

The aim is for improved understanding of environmental factors favoring the development of cancer of the uterine cervix.

Level of Effort: Professional Man-Years: 1/2

Serial No. NCI 501b

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A Review of the Scientific Literature on the Subject of
"Cancer in Man at the Site of Prior Benign Lesion of Skin or
Mucous Membrane"

Previous Serial Number: Same

Principal Investigator: Lucia J. Dunham, M.D.

Other Investigators: None

Man Years (July 1, 1972 - June 30, 1973)

Total:

Professional: 1/8

Other:

Project Description:

Objectives and Methods Employed: To categorize the prior benign lesions:
(1) Induced by known carcinogens (2) Benign lesions of a familial disease
(3) Congenital lesions (4) Benign tumors (5) Lesions based on trauma or
inflammation (6) Idiopathic lesions found clinically associated with cancer
(7) Lesions that are manifestation of a focal neuropathy or constitutional
disorder.

Major Findings: The types of injury and the pathology of the lesions
are noted and discussed. Risks of cancer are described by the type and site
of lesion.

Significance: To improve understanding of the natural history of some
human cancers and to provide suggestions for research studies from such
data.

Proposed Course: The project is completed.

Publications:

1. Dunham, L.J.: Cancer in man at site of prior benign lesion of skin
or mucous membrane: A review. Cancer Res. 32: 1359-1374, 1972

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-501b

Title: A Review of the Scientific Literature on the Subject of "Cancer in Man at the Site of Prior Benign Lesion of Skin or Mucous Membrane"

Principal Investigator: Lucia J. Dunham, M.D.

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: A review article entitled "Cancer in man at the site of prior benign lesion of skin or mucous membrane"

Approach: To categorize the prior benign lesions and the associated risks of cancer.

Progress: Article published in Cancer Research, July 1972.

Significance for Cancer Research (NCP Objective 5 Approach 2)

Level of Effort: Professional Man-Years: 1/8

Serial No. NCI 501g

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Histologically Diagnosed Cancers in Indians of the
United States, 1950 to 1966

Previous Serial Number: Same

Principal Investigators: Lucia J. Dunham, M.D., Gert L. Laqueur, M.D.,
and John C. Bailar, III, M.D.

Other Investigators: None

Man Years (July 1, 1972 - June 30, 1973)

Total:

Professional: 1/8

Other:

Project Description:

The histologic and site diagnoses of 694 cancers in USA Indians are reported and analyzed.

Publications:

1. Dunham, L.J., Bailar, J.C., III, and Laqueur, G.L.: Histologically diagnosed cancers in Indians of the United States, 1950 to 1966. J. Natl. Cancer Inst. (in press)

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-501g

Title: Histologically Diagnosed Cancers in Indians of the United States, 1950 to 1966

Principal Investigator: Lucia J. Dunham, M.D.; G.L. Laqueur; J.C. Bailar, III

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: Collection of data from a series of histologically diagnosed cancers in Indians of the United States, 1950 to 1966.

Approach: The histologic and site diagnoses of 694 cancers in USA Indians were reported and analyzed.

Progress: The report is in press; to be published in the JNCI in May, 1973.

Significance for Cancer Research (NCP Objective 5 Approach 1)

To improve understanding of cancer in a specific population group.

Level of Effort: Professional Man-Years: 1/8

Serial No. NCI 504

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: An Electron Optical Investigation of Normal and Pathologic Tissue

Previous Serial Number: Same

Principal Investigator: William G. Banfield, M.D.

Other Investigators: G. Kasnic, C.J. Dawe, W. Morgan, A. Farley, C.W. Lee, D. Small

Cooperating Units: Biological Laboratory, National Marine Fisheries Service (NOAA), Oxford, Maryland

Man Years (July 1, 1972 - June 30, 1973)

Total: 3/4

Professional: 1/2

Other: 1/4

Project Description:

Objectives: 1. To describe and interpret the ultrastructure of selected pathologic states and to compare the results with suitable controls.

2. To look for and study the inciting agent or accompanying agent in neoplastic and other disease states using human, animal and tissue culture material.

Major Findings: 1. A study of the dense granule occurring in the elementary body of the pox viruses remains to be written up.

2. The electron microscopy of an oyster herpesvirus was completed and published.

3. Tissue cultures as well as the primary tumor of a snake chondrosarcoma were examined for virus but none was found. However, a structure that may be in the class of the tubular structures described by Schaff and Grimley in tumors, certain virus infections, and "collagen diseases" was found in both the primary tumor and tissue culture but has disappeared from the tissue culture.

Significance: 1. The dense granule may be of fundamental functional importance in the development of the virus and in its effects on the host. There are indications that it may exist in other viruses outside of the pox group.

2. Herpesvirus has been linked with human lymphomas. Whether the oyster virus affects man must be determined.

3. The significance of the structure is not known, but if it can be induced to reappear in the tissue culture it can be studied under controlled conditions.

Proposed Course: 1. Write up the dense granule work. Attempt to isolate the granule for further study when time permits.

2. Attempt to induce the reappearance of the short tubular structures in the tissue culture of the snake chondrosarcoma.

Publications:

1. Farley, C.A., Banfield, W.G., Kasnic, G., and Foster, W.S.: Oyster herpes-type virus. Science 178: 759-760, 1972

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-504

Title: An Electron Optical Investigation of Normal and Pathologic Tissue

Principal Investigator: William G. Banfield, M.D.

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To find and study the inciting or accompanying agents or structures in neoplastic or other disease states.

Approach: Electron microscope and tissue culture.

Progress: No virus was found in tissue cultures or the primary tumor of a snake chondrosarcoma. However, a tubular structure probably of the class described by Schaff and Crimley in tumors, certain virus infections and "collagen diseases", was present in the cytoplasm. This structure disappeared from the cultures as has been the experience of others studying similar structures.

Significance for Cancer Research (NCP Objective 5 Approach 4)

The agents and structures present in the cancer cell must be known before they can be evaluated.

Level of Effort: Professional Man-Years: 1/2

Serial No. NCI 504b

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: An Investigation of the Collagenous Connective Tissues and Elastin

Previous Serial Number: Same

Principal Investigator: William G. Banfield, M.D.

Other Investigators: C.K. Lee, L. Sokoloff, W.C. Roberts, L.M. Buja, and C.W. Lee

Cooperating Units: NIAMD, Lab. Exp. Path., Section on Rheumatic Diseases;
NHI, Cardiology Branch, Section on Pathology

Man Years (July 1, 1972 - June 30, 1973)

Total: 1/2

Professional: 1/2

Other: 0

Project Description:

Objectives: To elucidate the normal and abnormal structure of elastin and collagen and their associated cellular and intercellular components.

Major Findings: 1. The results of the study of fibrous long spacing type collagen fibrils with especial reference to their occurrence in normal and diseased hearts is in press.

2. The connective tissues, elastin and collagen, are continuing to be studied with the electron probe and scanning electron microscope as indicated under those headings.

Significance: The mucoprotein collagen complex as expressed in the FLS type collagen fibrils may be an integral part of the connective tissue system. These fibrils are present, often in large numbers, in thyroid medullary tumors, Hodgkin's disease lymph nodes and other tumors. Their significance with respect to the metabolic state of the tissue in which they are found should be explored.

Proposed Course: Apply the scanning electron microscope, electron probe and ion probe to the analysis of connective tissues, particularly elastin and elastotic degeneration (senile elastosis).

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-504b

Title: An Investigation of the Collagenous Connective Tissues and Elastin

Principal Investigator: William G. Banfield, M.D.

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To elucidate the normal and abnormal structure of elastin and collagen and their associated cellular and intercellular exponents.

Approach: Transmission electron microscope and scanning electron microscope with X-ray analyzer.

Progress: Fibrous long spacing-type collagen is found in human myocardium particularly about vessels. A new type of collagen, "spiney" collagen, has also been described in the myocardium. Both are abnormally increased in rheumatic fever.

Significance for Cancer Research (NCP Objective 5 Approach 2,6)

Fibrous long spacing-type collagen is present in thyroid medullary tumors, Hodgkin's disease lymph nodes and other tumors. They may be significant with respect to the metabolic state of the tumor in which they are found.

Level of Effort: Professional Man-Years: 1/2

Serial No. NCI 505

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Evaluation of Potential Pulmonary Carcinogens, Asbestos, Tobacco, Silicates and Heavy Metals and their Relation to the Physiological Status of the Lung

Previous Serial Number: Same

Principal Investigator: Mearl F. Stanton, M.D.

Other Investigators: E. Miller, M. May, and B. Sanders

Cooperating Units: Johns-Manville Research and Engineering Center, Manville, New Jersey; Owens-Corning Fiberglass Corp., Toledo, Ohio; Dr. Fred Bock, Roswell Park Memorial Center for Cancer Research; Mel-Labs Inc., Contract NIH 70-2295

Man Years (July 1, 1972 - June 30, 1973)

Total: 1/2

Professional: 1/4

Other: 1/3

Project Description:

Objectives: (1) Investigations into pulmonary carcinogenesis with tobacco products and other potential pulmonary carcinogens for man. (2) Investigations into the factors affecting development of mesotheliomas experimentally induced with asbestos, fibrous glass and related fibrous minerals.

Major Findings: During 1972-73, two major interests of this unit that have been studied in the past year were continued. The final data have been collected on the initial series of experiments in which we induced epidermoid carcinoma of the lung in rats with cigarette condensate. Reasonably high yields have led us in collaboration with Dr. F. Bock to study the response of the rat lung to a series of cigarette condensate fractions which have varying potential for inducing papillomas on the skin of mice. These experiments employing approximately 1000 rats have begun and will require two and one-half years for completion. In addition, 18 monkeys have been implanted with pellets of beeswax, heptane soluble cigarette condensate or benzpyrene in collaboration with Dr. R. Adamson, Division of Cancer Treatment, NCI.

Secondly a large series of experiments concerning the response of the pleura of the rat to various fibrous carcinogens and related materials have

been initiated. Preliminary conclusions indicate that fibers greater than 3.0 microns are far less carcinogenic than fibers below this range. Further fibers of varying short lengths i.e., 3-10 microns are far less carcinogenic than longer fibers. These data apply equally to the asbestosis, glass and Al_2O_3 whiskers. These experiments are now taking several courses:

A. Establish the specificity of the carcinogenic response; B. Investigate diverse types of fibers (see below); C. Investigate other structural minerals and metals; D. Narrow the dimensional parameters relevant to the carcinogenicity of glass, asbestos and fibrous Al_2O_3 . These experiments all require some 2 - 2 1/2 years for completion. During this interim the experiments previously reported on preliminary data will serve as a fruitful source of several publications.

Proposed Course: A large-scale contract, funded by DCCP will be undertaken to test the screening ability of the pulmonary pellet method to detect differences between cigarette smoke condensate from cigarettes with high and low nicotine content. In our own laboratory, we intend to test the method with several innovative cigarette smoke condensates and to explore the system with smoke condensates of other types that are common air pollutants.

Briefly our plans call for testing various fractions of cigarette smoke condensate and the condensate of cigarette smoke from cigarettes with altered or reduced tar and nicotine content as well as extracts from specific air pollutants and other potential pulmonary carcinogens that may be lipid soluble. Work with fibers involves the tedious assessment of fiber sizes and separation of fibers into specific size groups for testing utilizing asbestoses, glasses, aluminum oxide, silica, iron and carborundum fibers. The effects of reduction in durability as a result of pyrolysis and the effect on surface properties by fiber coating will be investigated as time and space permit. Electron microscopy of candidate specimens of fibers and fine structure analysis of the early metaplastic alterations in pulmonary epithelium are in progress with the aim of understanding the preneoplastic changes that may be related to the induction of cancer.

In regard to fiber carcinogenesis, we do not plan on using present facilities as a screening process for the many and diverse materials that might have some potential for inducing cancer in man. We believe that such programs could best be carried out under other contracts or in other agencies. The major purpose of the experiments will be to elucidate the mechanism of fiber carcinogenesis and to determine its possible relationship to other types of carcinogenesis. The first step in this plan will be to gain a more precise assessment of durability and fiber sizes in the materials tested. If a relatively narrow range of fiber sizes can be incriminated, study of the effect of such fibers in vitro and in locations other than the pleura will be initiated. It is probable that fibrous glass will offer the most effective means of examining fiber carcinogenesis since it is relatively non-toxic and can be best handled in separation and isolation of relatively sized particles. However, in addition to the large series of experiments in progress with glass, the following materials are being tested: hydrated, partially hydrated and anhydrous fibrous gypsum, Al_2O_3 whiskers of 4 types, synthetic chrysotile to which cobalt has been added, glasses to which chromium has

been added, steel whiskers, tremolite and a series of fibrous talc products.

Publications:

1. Stanton, M.F., Miller, E., and Wrench, C.: Experimental induction of epidermoid carcinomas of the lung with cigarette smoke condensate. J. Natl. Cancer Inst. 49: 867-877, 1972
2. Stanton, M.F.: Some etiologic considerations of fiber carcinogenesis. In: Proceedings of the Working Group on Biological Effects of Asbestos. A Monograph Published by IARC, Lyon, France. Spring 1973

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-505

Title: Evaluation of Potential Pulmonary Carcinogens, Asbestos, Tobacco, Silicates and Heavy Metals and their Relation to the Physiological Status of the Lung

Principal Investigator: Mearl F. Stanton, M.D.

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To investigate mechanisms of carcinogens and detect potential carcinogens for the lung.

Approach: Various potential carcinogens such as asbestos, fibrous glass and durable whiskers of metal $< 3\mu$ in diameter are implanted in the pleura of rats.

Potential carcinogens such as cigarette smoke condensate and other liquid polycyclic hydrocarbons are implanted in the lung within beeswax pellets.

Histopathologic studies are done on all rats during the course of studies lasting 2-3 years. Fiber structure is quantitated.

Progress: The carcinogenicity of durable fibers depends on the dimensional properties of the fiber. Durable fibers below 2.5μ in diameter are far more carcinogenic than fibers of greater diameter. Fibers about 20μ in length are far more carcinogenic than fibers of shorter length. Cigarette smoke condensate induces pulmonary carcinomas by direct contact and in quantitative fashion related to dose.

Significance for Cancer Research (NCP Objective 1 Approach 1)

Asbestos and cigarette smoke are two of the few known carcinogens for man.

Level of Effort: Professional Man-Years: 1/4

Serial No. NCI 514

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Induced and Spontaneous Neoplasms in Laboratory Animals.
Factors that Influence their Incidence and the Chemical
Changes Induced by the Neoplasms

Previous Serial Number: Same

Principal Investigator: Ambadas S. Mulay, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1972 - June 30, 1973)

Total: 3/4

Professional: 1/2

Other: 1/4

Project Description:

A. Objectives: Induction of glandular stomach lesions in guinea pigs.

Methods Employed: A group of male guinea pigs of Hartley strain is given by stomach tube, suspension of nitrosomethyl urethane 3 times a week and another group receives the suspension by intraperitoneal route once a week. Animals are examined every day for their general health and possible initiation of tumors.

Major Findings: At the end of two years from the start of the experiments, no lesions were found in the stomach and all other organs appeared normal.

Significance: A reliable method of producing high incidence of gastric carcinoma will give us a tool to study the chemistry of the lesion and may be useful in determining relative effectiveness of various chemotherapeutic agents.

Proposed Course: No further work is planned on this project.

B. Objectives: Induction of glandular stomach lesions in rats.

Methods Employed: Male rats of Osborne-Mendel strain and male and female rats of Wistar strain have been started on N-methyl-N-nitroso-N-nitroguanidine in drinking water. Some groups of rats received sodium nitrite in addition to N-methyl-N-nitroso-N-nitroguanidine in their drinking water.

Major Findings: Very few stomach lesions were observed in rats receiving N-methyl-N-nitroso-N-nitroguanidine in their drinking water. Only 3 possible glandular stomach lesions were noted in over 100 rats examined. All rats receiving addition of sodium nitrite had many forestomach lesions including dyskeratosis acanthosis, hyperkeratosis and/or multiple plaques of thickened mucosa, visible grossly as raised gray areas. No glandular stomach lesions were noted in these animals.

Significance: Induction of glandular stomach tumors could lead to development of a transplantable stomach tumor. This could prove to be an interesting experimental tool.

Proposed Course: No further work is planned on this project.

C. Objectives: Induction of glandular stomach lesions in rats.

Methods Employed: Male rats of Osborne-Mendel strain are given by stomach tube 10 mg of N-methyl-N-nitroso-N-nitroguanidine in 1 ml of 5% DMSO per animal per week.

Major Findings: The experiment is still going. No animals have come to autopsy yet.

Significance: Induction of glandular stomach tumors could lead to development of a transplantable stomach tumor. This could prove to be an interesting experimental tool.

Proposed Course: Work will be discontinued when these experiments are finished.

D. Objectives: Induction of lung tumors in rats.

Methods Employed: Osborne-Mendel rats are tube-fed twice a week with freshly prepared solution of 4-nitroquinoline-1-oxide in 10% dimethyl sulfoxide. Rats are examined at regular intervals for signs of tumors.

Major Findings: No lung tumors were noted in any of the 35 rats examined after about 30 feedings and a year and a half after the start of the experiment. No other lesions were noted in these rats except 3 forestomach papillomas.

Significance: Transplantable lung tumors could be used as an interesting experimental tool.

Proposed Course: No further work is planned on this project.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-514

Title: Induced and Spontaneous Neoplasms in Laboratory Animals. Factors that Influence Their Incidence and the Chemical Changes Induced by the Neoplasms

Principal Investigator: Ambadas S. Mulay, Ph.D.

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To produce transplantable animal tumors to serve as models for human cancers.

Approach: Guinea pigs were given, by stomach tube, nitrosomethyl urethane 3 times a week and another group received the carcinogen by intraperitoneal route once a week. Rats were given N-methyl-N-nitroso-N-nitroguanidine in drinking water. Rats were given, by stomach tube, N-methyl-N-nitroso-N-nitroguanidine in DMSO, once a week. Rats were tube fed twice a week with a solution of 4-nitroquinoline-1-oxide, and they were examined at regular intervals for signs of tumors.

Progress: There were many forestomach tumors and some glandular stomach tumors. No satisfactory transplantable tumor developed.

Significance for Cancer Research (NCP Objective 1 Approach 4)

A transplantable gastric carcinoma will give us a tool to study the chemistry of the lesion and could be useful in determining relative efficacy of various therapeutic agents.

Level of Effort: Professional Man-Years: 1/2

Serial No. NCI 516c

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Viral Carcinogenesis. The Murine Sarcomagenic Viruses:
Problems of Immunity, Resistance, and Pathogenesis and their
Relation to Cell Structure

Previous Serial Number: Same

Principal Investigator: Mearl F. Stanton, M.D.

Other Investigators: E. Miller, M. May, and B. Sanders and staff

Cooperating Units: Lloyd Law, M.D. and staff (NCI 3200), Richard Adamson, M.D.
and staff (LCP)

Man Years (July 1, 1972 - June 30, 1973)

Total: 1/2

Professional: 1/4

Other: 1/3

Project Description:

Objectives: A study and accessioning of cancer in laboratory animals. Collection and development of competence in special areas of cancer morphology. Studies of histopathological and fine structure alterations related to the pathogenesis of disease induced by oncogenic viruses.

Major Findings: An ongoing program has been the development of special competence in the histopathological and fine structural aspects of tumors of the lung, bone and mammary glands of rodents. Collection and examination of induced and naturally occurring bone tumors has been in progress for more than 5 years and will this year lead to the publication of a chapter on bone tumors in mice in the current series of WHO monographs on the "Pathology of Neoplasms in Experimental Animals". Our interest in bone tumors stems from the induction of bone tumors in rodents with chemical carcinogens several years ago. A series of these tumors ranging from Ewing's type to osteogenic sarcomas are available as transplantable tumors and are frequently supplied to other investigators. Currently, Dr. Richard Adamson, Laboratory of Chemical Pharmacology is studying the effects of bone seeking heavy metal chemotherapeutic agents on the osteogenic varieties.

The collection and development of competence in lung tumors is essential for the work previously described and additionally serves as an aid to other

investigators in the area and to the NCI Registry of Experimental Cancers. A current project on which Ms. May serves is the study of the fine structure of the early squamous metaplasia preceding carcinoma at the site of implanted carcinogen pellets and the comparison of this change with the metaplasia associated with non-specific pulmonary inflammation which does not progress to cancer.

Interest in mammary neoplasia in rats is an offshoot of current long-term pulmonary experiments. Since over 50% of female strain OM rats develop mammary tumors in the second year of life it is necessary to surgically remove these in our experimental groups. As a result we have correlated data on incidence, growth rate and morphology in several hundred excised mammary neoplasms.

Our interest in the morphology and fine structure of tumors induced by murine sarcoma viruses continues along lines of previous publications. The natural history and origin of these tumors particularly those of bone in the rat have received exhaustive study. Recently interest has centered on the morphology of cultured mouse embryo cells of line 3T3 which following infection with MSV (Moloney) show modulations in structure to an epithelioid type with fine structural characteristics previously identified as unique hallmarks of infection with this virus.

Experience gained from examining neoplasms induced by murine sarcomagenic viruses strongly suggests that all of the neoplasms may have a common cell of origin. If this is the case, the most likely candidate would be a primitive cell of the reticuloendothelial-histiocytic series. Derivation of all of the neoplasms from cells closely related to lymphoreticular neoplasms would of course be most significant to the phenomenon of C-type virus oncogenesis.

As previously reported, the disease induced by this virus in both rats and mice is initially an inflammatory granuloma in which atypical cells arise. This response occurs in many tissues although definite tissue-selectivity occurs. Ultimately a small percentage of the lesions evolve into simple neoplasms. The unique reactive response to either the atypical cells or the murine sarcoma virus and its relation to leukemogenesis and such neoplasms in man (for example, Kaposi's disease, Hodgkin's disease and the reticuloendotheliosis) that show similar reactive components make this disease a particularly critical one in studies of viral carcinogenesis. Further studies in viral oncogenesis have centered on characterization of cell structure and cell alterations induced by infection with so-called "murine sarcoma virus" by electron microscopy.

Proposed Course: The current problem is one of seeking ways of clearly and unequivocally establishing the progenitor cell type of the neoplasms. No simple means is at hand and investigations may be deferred for some time unless a reasonable approach to this problem can be devised. Nevertheless, the electron microscopy, histology and distributional analysis of lesions from several hundred rats are being analyzed. Of particular interest is our effort to compare changes induced in the tumors of osseous and neurogenic tissues

with those in the transplantable MSB-1. Analysis of tissue morphology by a variety of means will probably occupy the better part of the year.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-516c

Title: Viral Carcinogenesis. The Murine Sarcomagenic Viruses: Problems of Immunity, Resistance, and Pathogenesis and their Relation to Cell Structure

Principal Investigator: Mearl F. Stanton, M.D.

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: Collection and development of competence in special areas of morphology.

Approach: Histopathologic and electron microscopic examinations are carried out on a variety of diseases and neoplasms induced by viruses in attempts to collate morphologic structure with etiologic agents and mechanism of action.

Progress: Studies with C-type virus induced lesions have been particularly fruitful in revealing certain structural features in all types of cells that seem to be hallmarks of viral invasion.

Significance for Cancer Research (NCP Objective 1 Approach 2)

Identification of hallmarks of viral infection might serve as a means of alerting to increased risk of cancer.

Level of Effort: Professional Man-Years: 1/4

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Induction of Neoplasms, Selection of Transplantable Strains
of Tumors and Study of Tumor and Host Chemistry

Previous Serial Number: Same

Principal Investigator: Ambadas S. Mulay, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1972 - June 30, 1973)

Total: 3/4

Professional: 1/2

Other: 1/4

Project Description:

A. Objectives: To induce glandular stomach tumors in rats and to establish a transplantable strain of these tumors.

Methods Employed: Male rats of Osborne-Mendel strain and male and female rats of Buffalo strain are fed a semisynthetic diet deficient in vitamin A. To this diet is added a carcinogen, 2,7-diacetamidofluorene, suspended in corn oil. Other groups of rats are fed the same diet except for the addition of an irritant - celite. There are control groups without carcinogen and without irritant. Diets are thoroughly mixed with mechanical mixer and stored at 0°C. Fresh diet is prepared every other week. Animals are regularly examined for their general health and possible tumors.

Major Findings: No glandular stomach tumors were noted in any of the animals. These animals were on experimental diet for 6 months and the experiments terminated a year later. Six hepatomas and two subcutaneous fibrosarcomas were noted in 34 animals examined.

Significance: This experimental study, when successful, may throw some light on the mechanism of induction of glandular stomach tumors. Successful development of transplantable tumor would give us a tool for further study of this neoplasm.

Proposed Course: No further work is planned on this project.

B. Objectives: To induce glandular stomach tumors in rats and to establish a transplantable strain of these tumors.

Methods Employed: Synthesize the chemical, 4-hydroxyaminoquinoline-1-oxide. Feed the compound in food, in water, or by stomach tube to Osborne-Mendel, Fischer, Marshall, and Wistar rats. Examine the animals for tumors at regular intervals. Transplant the primary tumors as they become available and try to establish a transplantable tumor line.

Major Findings: Most of the animals had forestomach lesions which included acanthosis, hyperkeratosis and plaques of thickened mucosa with or without depression in the center. No glandular stomach tumors were noted in the animals that came to autopsy.

Significance: Transplantable glandular stomach tumor could be an interesting experimental tool for studying the characteristics of this tumor.

Proposed Course: The work will be discontinued as soon as the experiment is finished.

C. Objectives: To induce stomach tumors in rats and to establish a transplantable strain of these tumors.

Methods Employed: A suspension of nitrosomethyl urethane in 20% ethanol is fed by a tube to Osborne-Mendel rats once every week. Animals are examined every day for their general health and periodically checked for signs of tumor induction on gross examination.

Major Findings: Forestomach tumors were noted in a majority of the animals examined. No glandular stomach tumors were found. Microscopic examination of the lesions has not yet been made.

Significance: Transplantable glandular stomach tumor could be an interesting experimental tool for the study of these tumors.

Proposed Course: Work will be discontinued when these experiments are finished.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-519

Title: Induction of Neoplasms, Selection of Transplantable Strains of Tumor and Study of Tumor and Host Chemistry.

Principal Investigator: Ambadas S. Mulay, Ph.D.

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To determine effectiveness of exogenous chemicals to induce cancer, find agents that modify the host response to induction of neoplasm, and study the changes in the host chemistry in response to neoplastic growth.

Approach: Various probable carcinogens are fed to animals to determine their effectiveness to induce neoplasm. Rats were fed semisynthetic diet deficient in Vitamin A, to which 2,7-diacetamidofluorene was added. Irritants were added to the diet of some groups and controls received no carcinogen. Rats were fed with tube, 4-hydroxyaminoquinoline-1-oxide, 3 times a week. Rats were fed with stomach tube, nitrosomethyl urethane, once every week. Animals were checked periodically for signs of tumor induction.

Progress: Many forestomach papillomas, subcutaneous fibrosarcomas, and liver tumors were induced. No transplantable tumors of glandular stomach or lung were developed.

Significance for Cancer Research (NCP Objective 1 Approach 3,4)

Transplantable animal tumors could serve as models for study of human cancer. Metabolic factors that may modify the course of the neoplasm could be studied on such models and efficacy of therapeutic agents could be determined on them.

Level of Effort: Professional Man-Years: 1/2

Serial No. NCI 521a

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Investigations of Mechanisms of Tumor Induction by Polyoma Virus in Mice

Previous Serial Number: Same

Principal Investigator: Clyde J. Dawe, M.D.

Other Investigators: Dr. Teruyo Sakakura (Aichi Cancer Institute, Nagoya, Japan); and W.D. Morgan

Cooperating Units: None

Man Years (July 1, 1972 - June 30, 1973)

Total:

Professional: 6/10

Other:

Project Description:

Objectives: To identify the conditions necessary for and the cellular activities leading to tumor development in mouse salivary glands infected with polyoma virus. Previously published works from this project have shown the following:

1. In PV-infected organ cultures of salivary glands, morphologic transformation occurs, closely resembling tumor development in vivo. When such transformed cultures are transplanted to mice, typical salivary gland tumors appear at the site of transplant.

2. In the organ culture system, the visible sequence of events is:
(a) Lytic infection of mesenchyme; (b) Lytic infection of epithelium;
(c) Emergence of transformed cells, some of which undergo viral lysis while others proliferate.

3. At very early stages of embryonic development, salivary gland or pre-salivary tissue does not undergo neoplastic transformation subsequent to PV infection.

4. When the epithelial and mesenchymal components of embryonic salivary glands are isolated from each other by trypsin dissection neither component is able to give rise to a neoplasm after infection with PV.

5. Recombination of salivary epithelium with salivary mesenchyme restores the capacity for tumor development to occur. Recombination of salivary epithelium with tooth bud mesenchyme, or of tooth bud epithelium with salivary gland mesenchyme, does not restore the capacity for tumor development.

6. PV-induced tumor tissue can substitute for normal salivary gland mesenchyme in supporting morphogenesis in vivo. In vitro, the tumor tissue has so far been observed to support growth, but not morphogenesis of salivary epithelium.

7. Epithelial origin of the PV-induced salivary gland tumors has been demonstrated. This was done by reassembling glands from T₆T₆ chromosome-marked epithelium and unmarked mesenchyme, then determining the presence or absence of the marker chromosomes in tumors induced by PV in the reassemblies.

Current Objectives:

1. In view of the above findings, an hypothesis has been constructed for testing. The hypothesis states that in the process of becoming neoplastic, epithelium develops the ability to produce for itself, in unrestricted quantities, the growth-promoting factors that it normally depends upon "proper" supporting mesenchyme to provide. This construction would explain the ability of PV-induced tumors to promote growth in normal salivary epithelium, just as normal salivary mesenchyme does.

One of the current objectives, therefore, is to determine whether polyoma tumors can also substitute for normal salivary mesenchyme in supporting tumorigenesis in salivary epithelium infected with polyoma virus. Positive results would constitute support for the hypothesis, but would not prove it.

2. If the hypothesis is correct, one would expect the PV-induced salivary tumor tissue to have the same degree of specificity for inducing growth and morphogenesis as normal salivary mesenchyme. A second objective, therefore, is to study the range of activity of the inducing factor present in polyoma salivary tumors.

3. The most direct and convincing evidence in support of the hypothesis would be isolation and characterization of the presumptive substance in polyoma salivary tumors that induces growth and morphogenesis in normal salivary epithelium. Isolation and characterization of this substance is contemplated, but has not yet been attempted.

Methods Employed: Isolated salivary epithelium from normal C₃H/Bi mouse embryos of 13 to 14-days' gestation is placed on one side of a millipore filter, while polyoma salivary tumor cells of line 9391 are grown on the opposite side. After 2 to 3 days, when the salivary epithelium has become attached and spread out to form a monolayer, polyoma virus is added to the culture. Cultures of this type are then carried for several months to follow the growth and morphological characteristics of the epithelium. Control cultures consist of similar configurations using normal salivary mesenchyme

opposite normal salivary epithelium, and mammary epithelium opposite salivary mesenchyme. Salivary epithelium alone is also used as a control, and all four of these types of culture will eventually be studied both with and without polyoma virus infection.

Evaluation of results is based on (1) microscopic examination of fixed-stained preparations of the trans-filter cultures and (2) transplantation of the salivary epithelial cells to syngeneic newborn mice to determine tumorigenicity and histologic characteristics of the tumors induced, if any. To be able to determine whether contamination by 9391 cells of the epithelium under test has occurred, we will use T₆T₆ chromosome-marked salivary epithelium. Tumors arising from this epithelium should carry the T₆T₆ markers, while those originating from line 9391 would not.

In (2) above, normal rudimentary epithelium from 16-day mammary rudiments or from 13-14 day salivary rudiments are placed within a small piece of transplantable salivary tumor and transplanted subcutaneously to syngeneic newborn mice. After 12-14 days, the transplants are removed and serial-sectioned to determine whether morphogenesis has occurred in the epithelium within the tumor.

Major Findings:

1. In absence of virus, normal salivary epithelium trans-filter to normal salivary mesenchyme grew well for periods longer than 1 month, but did not undergo morphological transformation. In the absence of trans-filter salivary mesenchyme, salivary epithelium failed to sustain itself beyond 2-3 weeks.
2. In the absence of virus, normal salivary epithelium trans-filter to 9391 tumor cells grew well for periods longer than 1 month, but did not undergo morphological transformation.
3. In 5 separate culture experiments, salivary epithelium placed trans-filter to normal salivary mesenchyme infected with polyoma virus underwent transformation and grew for periods of 3 months or more. Morphologically the transformed epithelium was identical with salivary epithelium transformed in organ culture or in vivo. Two of the trans-filter transformed lines have been kept growing continuously for periods of 5 and 8 months respectively. In one trans-filter experiment, transformed mesenchyme also emerged. Both the epithelial and the mesenchymal transformed cell lines produce polyoma virus, demonstrated by P₃₈₈ cell infectivity and by tumor-inducing activity in newborn mice. Typical polyoma-type intranuclear inclusion bodies are present in the transformed epithelial lines.

The trans-filter transformed epithelial cell lines grow very slowly and are difficult to grow on glass although they do well on millipore membranes. Apparently the polyoma carrier state results in a situation where cell proliferation rate barely exceeds cell lysis rate. One attempt to rid the cultures of polyoma virus by adding mouse anti-polyoma serum was unsuccessful. When

these cells can be grown in larger quantities, renewed attempts will be made to eliminate the virus, as well as to transplant the cells to syngeneic mice to test their tumorigenicity.

4. It was noted in last year's report that preliminary observations indicated that when polyoma virus is grown on salivary tumor cells in culture, the resulting virus strain shows an increased mammary tumor-inducing activity. This has been confirmed by a more extensive experience. Interestingly, it is further confirmed by the observation that virus grown in the carrier state on salivary epithelium transformed by PV in the trans-filter experiments also induces mammary tumors at a higher rate than the parent P₃₈₈-propagated strain.

Contrary to last year's report, the so-called mammotrophic strain of PV has not lost its salivary tumor-inducing activity. In all mice bearing mammary tumors, either gross or microscopic salivary tumors have also been found. The clinical picture remains different, however, in that mice inoculated with the mammotrophic strain often manifest mammary tumors before salivary tumors.

Methods of propagating polyoma virus to produce virus with differing tumor-inducing predilections are now known as follows:

1. Grown in vivo in newborn mouse kidney → High ameloblastoma incidence.
2. Grown in vitro on P₃₈₈ mouse lymphoma cells → High thymic epithelioma incidence.
3. Grown in vitro on 9391 salivary tumor cells . High mammary tumor incidence.
4. Grown in vitro on P₃₈₈ mouse cells using defatted cow's milk as protein source Loss of all tumor-inducing activity in mice, without loss of tumor-inducing activity in hamsters.
5. We have succeeded in repeating the observation that mammary gland rudimentary epithelium can be induced to undergo extensive morphogenesis by PV-induced salivary tumor, in vivo. Thus the specificity of salivary tumor tissue for activity in secondary induction systems corresponds to that of normal salivary mesenchyme as far as current information goes. Both normal salivary mesenchyme and salivary tumor tissue are able to induce morphogenesis in mammary gland epithelial rudiments as well as in salivary gland epithelial rudiments. Dr. Sakakura's experiments during the past year demonstrated that normal salivary mesenchyme recombined with mammary rudiment epithelium induces morphogenesis in vivo of a glandular structure that resembles salivary gland in its general configuration, but secretes material that microscopically resembles milk.

Significance: The requirement of epithelial-mesenchymal interaction for neoplastic transformation of salivary epithelium infected with polyoma virus has been re-emphasized by the results of the trans-filter experiments. These results suggest that the role of mesenchyme in supporting tumor development is similar to the one it plays in supporting normal morphogenesis, since normal morphogenesis can also be induced across the millipore barrier. A possible mechanism through which the mesenchyme acts may be through activation of a specific gene site that would otherwise not be receptive to the polyoma virus. Other possibilities such as viral transduction of a mesenchymal cell trait are not ruled out.

Repetition of the experiment showing that mammary epithelium can be induced to undergo morphogenesis within salivary tumor tissue establishes another point of comparability between normal salivary mesenchyme and PV-induced salivary tumor. This makes it seem more probable that the salivary tumors produce the same factor(s) needed for normal growth and morphogenesis of salivary gland. The information now available is consistent with the hypothesis that the mechanisms by which the salivary epithelial tumors become independent (autonomous) is through activation of a growth-supporting function that is normally present in salivary mesenchyme, but not in salivary epithelium. It is now essential to isolate and identify this factor or factors. When its chemical nature is known, it may be possible to devise chemical or immunological antagonists, and so to inhibit tumor growth.

The discovery of a way to grow a mammotrophic strain of polyoma virus makes it practical to use this virus to study mammary carcinogenesis in mice. No other virus, chemical, or physical agent is known to induce mammary tumors as rapidly as this virus, which, when inoculated at birth, causes mammary tumors to appear in virgin females at 2-3 months. The tumors are often multiple, sometimes arising in 4 different mammary glands simultaneously. They are histologically different from mammary tumors induced by MTV, and they occasionally appear in uncastrated males. Their hormone responsiveness has not been studied.

Proposed Course: Work on this project has been temporarily suspended while the laboratory is undergoing renovations. As soon as these are completed, work will be resumed on the trans-filter experiments. In the meantime, although our operations are strongly curtailed, we will continue efforts to grow in greater quantities the salivary epithelium transformed trans-filter from salivary mesenchyme. When back in full operation, we will press to complete other phases of this project, including transformation of salivary epithelium trans-filter from salivary tumor cells, with and without virus.

If technical assistance as well as collaborative support from a biochemist can be obtained, we should move ahead with attempts to isolate, concentrate, and identify the presumptive growth-promoting factor(s) in the salivary tumor tissue. This is probably a protein, polypeptide, or glycoprotein, and a logical first approach would be to use gel electrophoresis followed by tests of the bands for growth promoting activity when normal

salivary epithelium is seeded on them.

As an additional control and at the same time as a test for viral transduction, an experiment will be done in which virus grown solely on salivary mesenchyme will be used to infect salivary epithelium. If transduction is involved in the PV-salivary tumor system, such virus should be able to transform salivary epithelium in the absence of direct or trans-filter contact with salivary mesenchyme.

Publications:

1. Dawe, C.J.: Changes in cell interrelationships during epithelial carcinogenesis. In Nakahara, W., Takayama, S., Sugimura, T., and Odashima, S. (Eds.): Topics in Chemical Carcinogenesis. Tokyo, University of Tokyo Press, 1972, pp. 401-427.
2. Dawe, C.J.: Epithelial-mesenchymal interactions in relation to the genesis of polyoma virus-induced tumours of mouse salivary gland. In Tarin, D. (Ed.): Tissue Interactions in Carcinogenesis. New York, Academic Press Inc., 1972, pp. 305-358.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-521a

Title: Investigations of Mechanisms of Tumor Induction by Polyoma Virus in Mice

Principal Investigator: Clyde J. Dawe, M.D.

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To determine the influences of epithelial-mesenchymal interactions on the genesis of polyoma-induced salivary gland tumors in mice, and to identify the mechanisms involved.

Approach: Organ and cell culture methods are used to study polyoma virus-induced neoplastic transformation of mouse salivary gland epithelium. By using an enzymic dissection method, developmental stages of salivary glands from mouse embryos are dissected into epithelial and mesenchymal components. These components are then infected separately or after recombination with each other or with other cell types from other organs, and the response to polyoma virus is observed. In some instances, the various types of tissue components are placed in trans-filter confrontations with each other, and the response to polyoma virus is observed.

Progress: Transformation of salivary gland epithelium after infection with polyoma virus takes place only if the epithelium is maintained in direct or in trans-filter contact with salivary mesenchyme. It has been found that polyoma-transformed salivary epithelium is capable of supporting growth and morphogenesis of non-transformed salivary epithelium. The tumor tissue can thus substitute for normal mesenchyme in supporting an inductive organogenetic interaction.

Significance for Cancer Research (NCP Objective 3 Approach 1,3)

The results show that epithelial mesenchymal interaction is necessary for polyoma virus to transform salivary epithelium. The role of the mesenchyme is hence as important as that of the transformed cell in this particular system. The findings suggest that the mechanism by which salivary epithelium becomes neoplastic is by acquiring the ability to synthesize a growth-promoting substance normally produced and controlled by mesenchyme.

Level of Effort: Professional Man-Years: 6/10

Serial No. NCI 538

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A Study of Phylogenetic Aspects of Neoplasia

Previous Serial Number: Same

Principal Investigator: Clyde J. Dawe, M.D. (Project Officer)

Other Investigators: J.C. Harshbarger, W.G. Banfield, D. Small, and others

Cooperating Units: None

Man Years (July 1, 1972 - June 30, 1973)

Total:

Professional:

Other:

Project Description:

Objectives: The continuing major objective of this project is to perform comparative studies of neoplasia in invertebrate and lower vertebrate animals, in order to gain a comprehensive view of the biology of neoplasia throughout the various animal phyla. This is done by collecting neoplasms and studying their morphology and natural history. Laboratory studies concerned with induction and transplantation of neoplasms in lower animals are also conducted. Phenomena such as regeneration, dedifferentiation, autotomy, molting, metamorphosis, graft rejection, and so on are studied in relation to the biology of neoplastic disease. The project also includes collection of literature concerned with neoplasms of invertebrate animals.

Major Findings: During the past year the experimental activities in this project were curtailed. The reporting investigator has continued to provide consultative diagnostic service to the Registry of Tumors in Lower Animals, operated under contract with the Smithsonian Institution under the direction of Dr. Harshbarger. On February 1 of fiscal 1973 the laboratory space (Rm. B-17, Bldg. 8) in which experiments in connection with this project were being performed was converted for other uses by another laboratory, upon administrative request. An experiment to determine the carcinogenicity of ethyl nitrosourea in carp was therefore terminated. No experiments with aquatic animals are in progress. A small amount of field work (see below) was continued, and cell cultures from a chondrosarcoma of a corn snake have been continued.

A. Most of the reporting investigator's work in this project has been concerned with organizational, advisory, and educational activities. Among these were:

1. Participation on a Program Review team serving the Canadian National Cancer Institute. Following a site visit to the University of British Columbia in Vancouver, the reviewing group recommended funding a project to search for neoplasms in fish and marine invertebrates exposed to industrial wastes in the Vancouver area. The project will be carried out by Drs. Hans Stich and Alfred Acton and staff.

2. Organization and chairmanship of a symposium on "Environmental Carcinogens in Feral Aquatic Animals" at the XIth International Cancer Congress in Florence, October 20-26, 1974. The proposed program has been approved by the Program Committee of the Congress.

3. Organization and chairmanship of a symposium on Aquatic Animals as Indicators of Environmental Carcinogens, sponsored by the International Union Against Cancer. This meeting will be held at the Huntingdon Research Centre, Huntingdon, England, October 16 to 18, 1974.

4. Serving as one of three guest co-editors of a volume to be entitled Neoplasms in Aquatic Animals, in the series on "Progress in Experimental Tumor Research", edited by F. Homburger.

5. Participated in the Armed Forces Institute of Pathology's symposium on Fish Pathology, August, 1972. Presented (with J.C. Harshbarger) a paper on Neoplasms in Feral Fishes: Their Significance to Cancer Research.

6. Will be Rapporteur for a Workshop on Pathologic Effects of Chemicals on Aquatic Organisms, May 8-9, 1973, sponsored by the Environmental Protection Agency's Laboratory in Gulf Breeze, Florida.

7. Will be discussant at symposium on Animal Models for Biomedical Research: V. Invertebrates, on April 19, 1973, at annual meeting of FASEB.

8. Wrote chapter on Comparative Neoplasia for a textbook entitled Cancer Medicine, edited by E. Frei and J. Holland, for Lea and Febiger, publishers.

B. Two lines of investigative work have been continued. One is a study of a chondrosarcoma of a corn snake, in tissue culture. This is being done in collaboration with Dr. David Small of the Laboratory of Microbiology and Immunology of NIDR, and with Dr. Banfield of the Laboratory of Pathology, DCBD, NCI. The chondrosarcoma cells have now been carried continuously in culture for 26 months. We were unsuccessful in transplanting these cells to the chorioallantoic membranes of embryonated corn snake eggs. Virus particles have not been found by electron microscopy, and lattices of electron-dense cytoplasmic particles seen in the primary tumor and in early cultures are no

longer found. The observations suggest that the cultured tumor cells have lost one of the differentiated properties they possessed in vivo.

In a continuation of our search for further examples of cholangiomas in white suckers, we recently examined a collection of 30 large fish of this species, taken from the same lake (Deep Creek Lake, Md.) where these tumors were first observed. No liver tumors of any type were found, in contrast to the finding of 3 cholangiomas in 12 fish from the first sampling in 1963. Since no further tumors have been found in the several samples taken since 1963, the possibility exists that some carcinogenic factor has disappeared from the environment.

Significance: These investigative and service activities add to the broad base of knowledge of neoplasms in animals. They contribute to the descriptive phase of cancer research in lower animals, which has received little attention from the main body of cancer researchers. Probably the chief value of this type of information is to enable cancer investigators to grasp a fuller understanding of cancer biology and to see the problem of cancer in its full context rather than as an isolated disorder or group of disorders of importance only to man and the higher vertebrates.

These studies are also useful in providing data and materials that unforeseeably find uses in evaluating important theories that are proposed concerning neoplasia. For example, it has been contended by R.A. Good that the apparent paucity of neoplasms in invertebrate animals supports his theory that the thymic system of cellular immunity developed evolutionarily as a mechanism to protect against malignant adaptation of cells. Another example is the oncogene theory, elaborated by R. Huebner, according to which the RNA tumor viruses evolved far back in evolution because of survival advantages afforded the host. The collection of tumors in cold-blooded vertebrate and invertebrate animals offers opportunities to obtain evidence on this concept, by looking for C-type RNA viruses and their reverse transcriptases.

A more practical potential value of these studies is that, by revealing epizootics of neoplasia in particular animals in particular locations, they may lead to discovery of environmental carcinogens. A proven past example of this application is the occurrence of hepatomas in trout fed diets contaminated with aflatoxins.

Proposed Course: Dr. Small is preparing a report on the corn snake chondrosarcoma, in which we will include a description of the cell line. No immediate further work with this cell line is planned.

Collection and examination of white suckers from Deep Creek Lake will continue as long as State Sports Fisheries technicians continue to make samplings. Preparations have been made to perform analyses for various pesticides in the tissues of fish, if tumor-bearing specimens become available.

Publications:

1. Dawe, C.J., and Harshbarger, J.C.: Neoplasms in feral fish: **Their** significance to cancer research. In Ribelin, W. and Migaki, G. (Eds.): Fish Pathology. (in press)
2. Dawe, C.J.: Comparative neoplasia. In Frei, E. and Holland, J. (Eds.): Cancer Medicine. (in press)

CONTRACT RESEARCH SUMMARY

Title: A Study of Phylogenetic Aspects of Neoplasia (NCI-538)

Principal Investigator: John C. Harshbarger, Ph.D.
Name/Address: Director, Registry of Tumors in Lower Animals
Performing: The Smithsonian Institution
Organization: Washington, D.C. 20560

Contract Number: PH 43-65-1072

Starting Date: July 1, 1973

Expiration Date: June 30, 1974

Goal: To collect, examine, classify, and preserve neoplasms in cold-blooded vertebrate and invertebrate animals, and to study experimentally the development of tumors in lower animals.

Approach: The Principal Investigator directs the operation of a Registry of Tumors in Lower Animals. Specimens are acquired from personal field investigations or through submittal by other investigators. The specimens are examined grossly, histologically, and in some cases by electron microscopy. Diagnoses are established and the specimens are described. The world literature on tumors in lower animals is collected. Field investigations and experimental inductions of tumors in lower animals are carried out. Publications of the findings are made.

Progress: Numerous neoplasms not previously known to occur in lower animals have been collected, classified, and recorded. Epizootics of certain tumors in marine mollusks and in several species of fish have been recognized, and environmental factors to explain them are being sought.

Significance for Cancer Research (NCP Objective 1 Approach 1,4)

Field studies and anatomical studies indicate environmental carcinogens that may be of importance in human cancer epidemiology or may be useful in designing analytical experiments to determine mechanisms of tumorigenesis.

Project Officer: Clyde J. Dawe, M.D.

Program: -

Site Visit Date: -

Technical Review Group:

Relevance Review Group: DCBD Contract Review Board (April 20, 1972)

FY73 Funds: \$82,769

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electron Probe and Scanning Electron Microscope

Previous Serial Number: Same

Principal Investigators: William G. Banfield, M.D., Stephen Doty, Ph.D.

Other Investigators: K. Heinrich, and staff of the National Bureau of Standards, C.W. Lee, H. Tipton (Biomedical Engineering and Instrumentation Branch, Division of Research Services), C.W. Berard, E.S. Jaffe, R.C. Braylan

Cooperating Units: National Bureau of Standards

Man Years (July 1, 1972 - June 30, 1973)

Total: 1/2
Professional: 1/2
Other: 0

Project Description:

Objectives: Application of electron probe and ion probe to the analysis of biological specimens.

Major Findings: 1. Dr. Doty, Mr. Lee and Dr. Banfield have developed a method for routinely obtaining frozen sections of tissue thin enough for conventional transmission electron microscopy.

2. Dr. Doty is supported by a special NCI fellowship under the joint sponsorship of Dr. Banfield, NCI and Dr. Heinrich of the National Bureau of Standards. We are proceeding with the quantitative analysis of elements, especially sulfur, in the epidermis, squamous cell carcinomas and basal cell tumors using the electron probe in Dr. Heinrich's laboratory of the National Bureau of Standards and the energy dispersive X-ray analyzer on the ETEC scanning electron microscope, Laboratory of Pathology, NCI. The initial effort is in working out the best method for preparing and introducing tissues into the scanning electron microscope for energy dispersive analysis and in developing standards.

3. A light microscope stage for the ETEC scanning electron microscope, primarily for the study of cathode luminescence, is almost off the drawing board.

Significance: 1. The preparation of ultrathin frozen sections allows for localizing the electron beam in the electron probe using transmission scanning. It also allows for immunological procedures and enzymatic reactions that cannot be done on fixed tissue. In addition, the ability to prepare frozen sections for conventional transmission electron microscopy opens the way for assessing artefacts introduced by fixation, dehydration and embedding.

2. Once a technique is developed for the quantitative elemental analysis of biological specimens on a cell to cell basis, using the electron probe, the technique can be used to correlate structure and metabolic state with chemical composition. If the ion probe can be used, molecular structural information can be obtained.

Proposed Course: 1. In collaboration with the National Bureau of Standards, continue to work out quantitative analytical methods for biology using the electron probe and ion probe.

2. Apply the scanning electron microscope, electron probe and ion probe to the analysis of connective tissues, particularly elastin, and to the analysis of stratified squamous epithelium, squamous carcinomas and basal cell tumors with especial emphasis on sulfur.

3. With Dr. Berard, Dr. Jaffe, and Dr. Braylan, use the ion probe and attempt to use the electron probe for the localization of immunological reactions to specific cells of the lymphoreticular system.

4. Repeat the work of Dr. K.G. Carroll who reported Ti and Zn in leukemic leukocytes and of Dr. D. Beaman who refuted Dr. Carroll's results. We will use the ion probe which is much more sensitive than the electron probe and analyzes as it dissects through the thickness of the cell.

5. Use the electron probe to analyze fibers used in Dr. Mearl Stanton's experiments in the production of tumors. In collaboration with Dr. Stanton, the scanning electron microscope will be used for sizing the fibers which he uses in his experiments. Many of these are in a size range beyond the resolution of the light microscope.

6. Use the light microscope stage constructed for the scanning electron microscope in a study of the cathode luminescence of elastic fibers.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-560

Title: Electron Probe and Scanning Electron Microscope

Principal Investigator: William G. Banfield, M.D., Stephen Doty, Ph.D.

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To perform a quantitative chemical analysis of cells and cell organelles in situ in tissue on a cell to cell basis.

Approach: Instruments: Electron probe X-ray microanalyzer, scanning electron microscope with X-ray analytical attachments, ion probe. Methods of specimen preparation are being developed which will give the greatest analytical sensitivity and which will be suitable for the application of several approaches to quantitative analysis. The approaches include Si absorption for the measurement of mass, Hall's thin fiber technique using the X-ray continuum for measuring the mass, and the Monte Carlo method for measuring specimen thickness.

Progress: A method has been developed for routinely obtaining frozen sections of tissue thin enough for transmission electron microscopy. This will make possible the preparation of tissue specimens for analysis with minimal loss of soluble constituents and immunologically or other active centers.

Significance for Cancer Research (NCP Objective $\frac{5}{(A \& B)}$ Approach $\frac{2,3}{\& 6}$)

This is an attempt to compare the chemical composition of cancer or pre-cancer cells with adjacent cells which are apparently normal in an attempt to find some objective criterion of malignancy.

Level of Effort: Professional Man-Years: 1/2

Serial No. NCI 561

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Collaborative Research and Other Activities

Previous Serial Number: Same

Principal Investigator: Mearl F. Stanton, M.D.

Other Investigators: None

Cooperating Units: JNCI, DCCP

Man Years (July 1, 1972 - June 30, 1973)

Total: 1/2
Professional: 1/2
Other: 0

Project Description: (Other Activities)

Fifty percent of the normal work week is spent as Scientific Editor of JNCI. Additional time in the normal work week is spent in Comparative Pathology Contract with the Smithsonian Institution, long-term animal holding facility contract with Melpar, Inc., and other contracts relevant to NCI 505.

Proposed Course: These activities will probably continue. In addition, time will be devoted to training in electron microscopy, preliminary investigations in the study of neoplasia in lower forms of animal life, and preparation of a monograph on bone tumors in mice.

Serial No. NCI 584

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Effects of Cyclic AMP on Mouse Salivary Gland Development
In Vitro

Previous Serial Number: Same

Principal Investigator: Clyde J. Dawe, M.D.

Other Investigators: W.D. Morgan, G. Johnson, and I.H. Pastan

Cooperating Units: None

Man Years (July 1, 1972 - June 30, 1973)

Total:

Professional: 3/10

Other:

Project Description:

Objectives: The nucleotide adenosine-3',5'-cyclic monophosphate is known to be a mediator of control of a number of cellular processes, among the most notable being responses to hormones and release of neurotransmitter substances. Another effect of cyclic AMP, recently reported by members of this group of collaborators, is to decrease the motility of certain connective tissue cells (L-929) in subconfluent cultures. Further, it has been found that associated with contact inhibition of movement and growth of 3T3 cells there is a rise in endogenous cyclic AMP levels. In general, cyclic AMP appears to influence growth and/or motility of connective tissue cells in culture, but has little directly discernible effect on isolated epithelial cells under comparable conditions.

As salivary gland organogenesis has been shown to depend on tissue-specific interaction between salivary mesenchyme and salivary epithelium, it follows that substances capable of altering the biologic function of the mesenchymal component should also alter the overall developmental process. Since cyclic AMP alters motility and growth of cells from certain mesenchymal cell lines, it might be expected to alter these parameters of salivary mesenchyme and indirectly to alter salivary gland development. These experiments were designed to test this postulate.

Methods Employed: The organ culture system previously used by us in studies of polyoma virus-induced transformation in salivary glands in vitro was adapted to this study. Submaxillary salivary rudiments are excised from

13- to 15-day mouse embryos of strain C₃H/Bi, and embedded in chicken plasma clot on coverslips in Leighton tubes. Cultures are maintained in 1.0 ml Eagle's Minimal Essential Medium, with daily complete replacement of the liquid phase. The substances under test include dibutyryl cyclic AMP, alone and in combination with theophylline (a potentiator of dbcAMP); theophylline alone; epinephrine (which induces changes in endogenous cAMP); isoproterenol, a catecholamine that induces increased endogenous cAMP in heart muscle and increased DNA synthesis and hyperplasia in murine salivary glands; prostaglandin E₁, which alters endogenous cyclic AMP levels, usually upwards; insulin, both alone and in combination with prostaglandin E₁; and ethyl alcohol (control), the vehicle for prostaglandin E₁.

Cultures are carried for 18-24 hours before subjecting them to test substances renewed daily for the subsequent 5 days. Daily observations are made on the progress of development of the rudiments, and photographs of representative changes in test and control cultures are made. In some runs, daily counts of the numbers of terminal buds on the developing adenomeres are made. It is planned to make low-magnification time-lapse cinematographic recordings of selected response patterns, to be compared with similar recordings of normal gland development by the split-frame technique devised by Morgan. Histologic and histochemical studies are performed on serial sections of test and control glands at selected intervals during the 5-day exposure or at termination of the run. Whole-mount stained preparations of representative samples of test and control glands are made to supplement the sectioned material and the photographic record of living cultures.

During the past year some preliminary observations were also made on the effects of dbcAMP on 2 polyoma-induced salivary tumors in primary cultures and on one such tumor in continuous cell culture. Control cultures consisting of primary explants of a spontaneous mouse mammary tumor, of a continuous cell line from a spontaneous mammary tumor, of a fibroblastic tumor cell line from a polyoma-induced fibrosarcoma, and of human and monkey tumor cell lines were also tested.

Major Findings: Dibutyryl cyclic AMP has produced marked alterations in salivary gland development, duplicated in kind, though lesser in extent, by prostaglandin E₁ and theophylline. Insulin has shown a slight inhibitory action against the effects of prostaglandin E₁, but has not yet been tested for inhibition of the exogenous dbcAMP action.

The most striking gross effect of these substances was cystic dilatation of the proximal salivary duct systems, both in the submandibular gland and in the sublingual gland, which was usually included in the explants. Duct dilatation was generally evident after the first 24 hours of exposure to dbcAMP, and by the 3rd or 4th day was maximal. By that time most dbcAMP-exposed rudiments could be grossly seen as spheroidal, loculated sacs filled with water-clear fluid. There was a tendency for rudiments with the most pronounced cystic change to come loose from the coverslips and drift in the medium as fluid-filled sphericles.

Concomitant with duct dilatation, there was a moderate decrease in rate of duct branching and formation of new terminal buds. This effect, however, was often difficult to evaluate in living cultures because cystic change rapidly obscured the visibility of terminal parts of the adenomeres. In the dbcAMP and prostaglandin E_1 -treated cultures, there frequently appeared a monolayer outgrowth of epithelium extending from the ostium of the duct where it was in contact with the coverslip. Such epithelial outgrowths also occasionally emanated from peripheral parts of the epithelial tree. Epithelial outgrowths of similar type were less frequent and less extensive in the control cultures.

Associated with these changes in the epithelial tissue component were alterations in the mesenchyme in dbcAMP- and prostaglandin E-treated rudiments. The corona of mesenchymal outgrowth on the coverslip at the base of attachment of the rudiment was slightly smaller in diameter than in controls, and the mesenchymal cells were less compactly arranged, forming a looser meshwork than in control cultures. There was also a tendency for mesenchymal cells to be more elongated, with long, sharp-tipped bipolar cytoplasmic processes, and to be arranged on the periphery in a more distinctly radial pattern. The mesenchymal cells in treated cultures tended to contain cytoplasmic vacuoles or granules at either end of the elongated nucleus, whereas the untreated mesenchymal cells had more uniformly clear, refractile cytoplasm.

The lumens of the dilated duct systems appear completely empty, except for some cellular debris normally to be expected. This suggests that the secreted material presumptively responsible for the duct dilatation contains little protein and is highly water-soluble. It has not yet been determined whether the duct dilatation results from hypersecretion, duct obstruction, or a combination of the two. In fixed whole mounts the ostia of the ducts appear to be open. However, we have no information regarding the possibility that contractility of periductal smooth muscle may be impaired. Time-lapse studies may answer this question. There is also a possibility that function of the paraductal parasympathetic ganglion may be involved. Histologically the neurones of these ganglia do not appear abnormal, but they have not been examined by special neurocytologic techniques.

It is our tentative impression that in dbcAMP-treated rudiments there is precocious appearance of mucoprotein secretory activity in cells of the terminal buds of the adenomeres. Cells filled with (presumptive) mucoprotein are present in these buds, and are numerous in occasional foci. Such foci have not been seen in control rudiments, but more extensive study is required.

Quantitative evaluation of the effects of dbcAMP on proliferative activity of epithelium and mesenchyme have not been made, but it can at least be stated that mitoses in both tissues are numerous, as they are also in controls. Possibly there is an increase of mitotic index in the epithelial sheets growing out from the proximal ends of the ducts. Tripolar mitoses and anaphase figures with lagging chromosomes are quite commonly present.

With respect to the effect of dbcAMP on primary explants of polyoma-induced salivary tumors, it was observed that there was a sharp decrease of mitotic figures in comparison with untreated controls. This was true for a predominantly epithelioid type salivary tumor as well as for a predominantly mesenchymoid type salivary tumor. In the tests using established cell lines, the mitosis-reducing effect of dbcAMP was much less marked, even questionable.

Significance: While this study has been in progress, a report from elsewhere (Defendi et al, Wistar Institute) states that in the presence of cyclic AMP, the transforming efficiency of polyoma virus acting on 3T3 mouse cells is as much as 1000-fold higher than in control cultures. In view of our findings cited above, the possibility is suggested that cyclic AMP may also influence the transformability of salivary epithelium in the organ culture systems we have used in the past. If so, antagonists of cyclic AMP could be expected to reduce the oncogenicity of polyoma virus, and it would obviously be useful to know of any agent that reduces the oncogenic response to a tumor virus.

Since cyclic AMP suppresses mitotic activity in polyoma tumors in vitro, it is indicated to pursue this observation further by determining whether agents capable of increasing cyclic AMP in vivo may inhibit polyoma tumor growth in vivo.

Proposed Course: Some of the experiments need to be repeated and the observations quantitated more extensively, particularly those on the decreased mitotic activity in primary salivary tumor explants in presence of dbcAMP. When this has been accomplished a report for publication will be prepared. Probably at that point a pilot study will be done to determine the effect of dbcAMP on tumorigenesis in salivary gland in organ cultures infected with polyoma virus.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-584

Title: The Effects of Cyclic AMP on Mouse Salivary Gland Development
In Vitro

Principal Investigator: Clyde J. Dawe, M.D.

Section: Comparative Oncology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To determine the effects of dibutyryl cyclic AMP on development of embryonic mouse salivary gland, and on polyoma virus-induced tumors of these glands.

Approach: Organ cultures of normal salivary gland rudiments and tissue cultures of salivary gland tumors are studied in presence and absence of dibutyryl cyclic AMP.

Progress: Cyclic AMP causes marked developmental anomalies in organ-cultured salivary glands. It also causes a reduction of mitotic activity in primary explants of salivary gland tumors.

Significance for Cancer Research (NCP Objective 3 Approach 3)

The findings suggest that cyclic AMP at concentrations used has a marked effect not only on normal developmental processes, but also on neoplastic growth. How this regulatory substance acts is subject of further investigations.

Level of Effort: Professional Man-Years: 3/10

Serial No. NCI 588

1. Laboratory of Pathology
2. Comparative Oncology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: In Vitro Culture of Articular Chondrocytes

Previous Serial Number: None

Principal Investigators: Willie D. Morgan and Leon Sokoloff, M.D.

Other Investigators: C.J. Malemud, V.M.L. Srivastava

Cooperating Units: Laboratory of Experimental Pathology, National Institute
of Arthritis, Metabolism and Digestive Diseases

Man Years (July 1, 1972 - June 30, 1973)

Total:

Professional:

Other:

Project Description:

This project was completed in the fall of 1972. It included studies of cell motility in rabbit articular chondrocytes, using the techniques of time-lapse photography. The project was a collaborative effort with Dr. Leon Sokoloff et al., who did radioassay and tissue culture studies on these cells in an effort to distinguish them from rabbit fibrocytes. The time-lapse work was carried out in our laboratory; while the culture work was done in the Laboratory of Experimental Pathology, by Dr. Sokoloff et al. In this report only the time-lapse work will be described.

Objectives: Articular chondrocytes can be distinguished morphologically from fibrocytes, but the distinction is not easily recognized. In monolayer cultures articular chondrocytes very much resemble fibrocytes. This morphological resemblance led Schubert and Hamerman to imply that connective tissue cells from a wide variety of sources come to behave like fibroblasts in monolayer culture. Fortunately, culture techniques have improved to a point where the culturing of articular chondrocytes is done routinely. And concomitant with improvements in chondrocyte culturing techniques, improvements in time-lapse microphotographic techniques were also made so that now we are able to make comparative cinematographic records of both rabbit articular chondrocytes and fibrocytes growing in monolayer cultures. The object of this project was to study cell motility in monolayer cultures of articular chondrocytes and cutaneous fibrocytes from young New Zealand rabbits, using techniques of time-lapse cinematography.

Methods Employed: Separate suspensions of articular chondrocytes and cutaneous fibrocytes (supplied by Dr. Leon Sokoloff) from New Zealand rabbits were put into separate modified multipurpose tissue culture chambers. The cells were then examined microscopically and diluted (by inspection) by flushing cells from the chamber by alternately filling the chamber with fresh medium and withdrawing it until not more than 50 cells per low power (X 33) microscopic field remained. The chambers were then placed on the stages of inverted Zeiss microscopes in a dual time-lapse photographic apparatus where the cultures were photographed at 1 minute intervals for 3 days. Some chondrocyte and fibrocyte cultures were photographed side by side on 16 mm Plus X film. This technique permits maximal comparison when the processed films are analyzed. All cultures were fed with Dulbecco's medium with 10% fetal calf serum. Motion analysis was made with the aid of a Photo Data Analyzer.

Major Findings: Comparison of motion paths made by individual articular chondrocytes and cutaneous fibrocytes of young rabbits shows that the chondrocytes are about a third as motile as the fibrocytes. Four hour tracings of these cells reveal the motility of the chondrocytes to be approximately 0.08 $\mu\text{m}/\text{min}$ compared to 0.25 $\mu\text{m}/\text{min}$ for the fibrocytes.

Significance: The finding that rabbit articular chondrocytes are less motile than rabbit cutaneous fibrocytes indicates that these cells are distinguishable on the basis of their rates of motility. This finding suggests that perhaps other cells of questionable origin, condition, or function may be distinguished on the basis of motility, using time-lapse photographic techniques.

Proposed Course: The report of the findings of this collaborative project is in press. The motilities of several established cell lines, primary cell cultures, and explants--treated and untreated with tumor viruses or compounds, such as cyclic AMP--will be determined in future projects, employing the above described time-lapse photographic techniques.

Publications:

1. Sokoloff, L., Malemud, C.J., Srivastava, V.M.L., and Morgan, W.D.:
In vitro culture of articular chondrocytes. Fed. Proc. (in press)

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 588

Title: In Vitro Culture of Articular Chondrocytes

Principal Investigator: Willie D. Morgan, and Leon Sokoloff, M.D.

Section: Comparative Oncology (W.D. Morgan)

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To perform comparative studies of cell motility in rabbit articular chondrocytes and cutaneous fibroblasts.

Approach: Articular chondrocytes and cutaneous fibroblasts from 2 month old New Zealand rabbits were cultured separately in modified multipurpose tissue culture chambers. Three days of comparative photographs, including side by side (split frame) photographs of these cell types were made, one every minute, on 16 mm Plus X film. Motility of individual cells was determined with the aid of a Photo Data Analyzer.

Progress: Rabbit cutaneous fibroblasts are more than three times as motile ($0.25 \mu\text{m}/\text{min}$) as rabbit articular chondrocytes ($0.08 \mu\text{m}/\text{min}$).

Significance for Cancer Research (NCP Objective 5B Approach 4)

Level of Effort: Professional Man-Years:

Serial No. NCI 852

1. Laboratory of Pathology
2. Cytopathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Exfoliative cytology applied to human diagnostic problems and research problems

Previous Serial Number: Same

Principal Investigator: Elizabeth W. Chu, M.D.

Other Investigator: None

Cooperating Units: None

Man Years (July 1, 1972 through June 30, 1973)

Total: 12

Professional: 8

Other: 4

Project Description:

Objectives:

The primary responsibility of the Cytopathological Section is to provide accurate and complete diagnostic exfoliative cytology as well as to collaborate in various clinical research areas evaluating drug and radiation effects, hormonal status of patients when requested and status of cancer lesions. To accomplish these objectives continual improvement of existing techniques and innovation of new ones are the principal goals of this laboratory.

The specific objectives include:

1. Development of methods for automation of cytology (separate report to be submitted by Dr. Chester Herman).
2. Work to improve methods for cancer diagnosis.
3. Evaluation of aspiration cytology method applied to various sites.
4. To develop cytologic criteria for aspiration cytology diagnosis.
5. Work to improve chromosomal examination for cytogenetic analysis.
6. Evaluate and/or develop methods for individual chromosomal identification.
7. Evaluate the effects of prolonged use of cyclophosphamide on the urinary tract epithelium.

Methods Employed:

1. Standard Papanicolaou staining procedure is applied to preparations of various kinds of patient material. The specimens are then screened for abnormal cellular changes.

2. Special techniques involving the use of millipore filtration and special staining are required in many instances.

3. Cultures of peripheral blood lymphocytes, bone marrow cells and tissue biopsies are routine diagnostic techniques required in patients with hormonal, developmental and malignant problems.

Patient Material:

The figures compiled below are for the last twelve months.

March 1972 - February 1973

Type of Material Examined:	<u>Accessions</u>	<u>Smears</u>
Vaginal and Cervical	1,388	2,387
Other (Ascitic, pleural, gastric, prostatic, urine, bronchial washings, sputa, spinal fluids, sex chromatin, wound washings, breast, oral, aspirations, chromosome analysis, chromosome tissue analysis, and spinal procedures)	3,034	7,100
Total	<u>4,422</u>	<u>9,487</u>
Institutes	<u>Accessions</u>	<u>Smears</u>
NCI	2,166	4,507
NHLI	393	962
NIAMD	793	1,178
NIAID	262	799
NINDS	116	271
NIMH	94	199
NIDR	158	223
NEI	21	67
NICH	356	1,104
Referred	44	136
CC (EHS)	<u>19</u>	<u>41</u>
Total	<u>4,422</u>	<u>9,487</u>

Major Findings:

Major contributions of the Cytopathology Section:

1. Providing early diagnosis of neoplasms of the cervix, etc. and metastatic lesions of various sites not oftentimes previously diagnosed.
2. Establishing the definitive usefulness of aspiration cytology as instrumental in providing early and rapid diagnosis.
3. Providing chromosomal analysis for clinical studies.
4. Providing chromosomal banding techniques where recognition of individual chromosomes is of great research interest.
5. Providing cytologic methods to the research projects.
6. Cytologic evaluation of therapeutic effects on the urinary tract epithelium.

Significance:

1. The diagnostic value of exfoliative cytology in the clinical management of cancer is well established. The pilot study of aspiration cytology technique has proven its usefulness in establishing definitive diagnosis thereby eliminating the more complicated and lengthy procedure in the preparation of patient for an incisional biopsy.
2. There is a definite need for cytogenetic study especially in recognizing and analyzing individual chromosomes in both service and research fields.

Proposed Course:

The primary purpose is to provide a complete and prompt cytopathologic service for the entire staff of the clinical center in various research projects and the development of new and improved techniques within the laboratory is our important forward looking program.

Work in Progress:

1. In collaboration with Dr. Robert Aptekar (Arthritis and Rheumatism Branch) patients an I.V. cyclophosphamide will be studied for possible deleterious changes of the urinary tract.
2. In collaboration with Dr. Arnold Dellon of the Surgery Branch, a study is being made on patients with oral cancer to see whether cancer cells will be seen in bronchial washings in the absence of lung metastasis.
3. In collaboration with Drs. N. Cummings and B. Thach of the Dental Institute patients with Behcet's aphthae are being studied to see the relationship if any between the rise and fall of oral mucosal ulcerations and hormonal effects.
4. To study the sputum samples (and bronchial washings in selected cases) of the patients who have undergone upper body radiation therapy and also patients on chemotherapy to see if there are epithelial changes attributable to therapies.

Publications:

Soloway, M. S., Myers, G. H., Marrone, J. A., DelVecchio, P. R. and Malmgren, R. A.: Evaluation of urinary cytology as an indicator of bladder neoplasia in mice. Journal of Urology, 109: 249-252, 1973

Aptekar, R. G., Atkinson, J. P., Decker, J. L., Wolff, S. M. and Chu, E.W.: Bladder toxicity with chronic oral cyclophosphamide therapy in non-malignant disease. Lancet (in press)

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-852

Title: Exfoliative Cytology Applied to Human Diagnostic Problems and Research Problems

Principal Investigator: Elizabeth W. Chu, M.D.

Section: Cytopathology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: 1. To provide complete and accurate diagnostic cytology and collaborate in research areas of therapeutic hormonal and radiation effects. 2. To provide chromosomal analysis for medical cytogenetic studies.

Approach: 1. Standard Papanicolaou staining, filtration technique and special staining are the methods employed. 2. Peripheral blood as well as tissue cultures are done and chromosomes stained by Giemsa and also banding techniques.

Progress: 1. Ca-in-situ has been detected in totally unsuspected patients. 2. Abnormal cell quantitation by filtration method assists in patient therapy. 3. Erratic karyotypes and mosaicism have been reported. Banding staining in assessing individual chromosomes is now in progress.

Significance for Cancer Research (NCP Objective 1 Approach 1,2,
& 3)

Level of Effort: Professional Man-Years: 1

Serial No. NCI 854

1. Laboratory of Pathology
2. Cytopathology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Experimental studies on carcinogenesis, endocrine factors and cytogenetic characters

Previous Serial Number: Same

Principal Investigator: Elizabeth W. Chu, M.D.

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1972 through June 30, 1973)

Total: 1

Professional: 1

Other: None

Project Description

Objectives:

1. To study the pathogenesis of lesions of rodents after treatment with chemical and biological agents and to study the influence of these agents in the development of neoplasms.

2. To study the effects of Enovid in rodents.

3. To study the effects of Trichomonads in rodents.

4. To study the effects of Cytosan in rodents.

5. To study chromosomal expressions of spleen cells from splenectomized patients with lymphoma.

Methods Employed:

1. Hamsters of four-weeks age were treated with:

(a) 0.05% DMBA in ground purina was given from Friday 4:00 p.m. to Monday 8:00 a.m. Tap water to drink.

(b) 0.05% DMBA in ground food as in (a) but 10% alcohol as daily drinking water.

(c) No DMBA put in their food but 10% alcohol to drink.

2. Mice (Balb/c) are fed with Enovid pellets. At end of one year's treatment, the cervical lesions will be transplanted to another animal.
3. Mice (Balb/c).
 - I.V. Cytosin (0.1 mg/gm. body weight)
 - I.V. Cytosin (0.2 mg/gm. body weight)
 - Mice will receive I.V. 0.2 mg/gm. body weight at monthly intervals.
 - Daily urine samples will be collected and screened for G-U tract epithelial changes.
4. Spleen tissues from lymphoma patients are minced finely and the cell suspensions are processed for chromosomal analysis.

Major Findings:

1. A pilot study on effects of alcohol in the promotion of tumor revealed that 10% alcohol increased the incidence of cancer production by DMBA in hamsters. Experiments for the purpose to reproduce the results are nearing its final lap.
2. All mice that were fed with Enovid pellets developed vaginal cervical lesions suggestive of cancer. Experiments are now underway to reproduce the lesions for transplantation thereby settling the question of true cancerous state.
3. From the patient study here at NIH that Cytosin may produce epithelial changes mimicking cancer but the changes are reversible upon withdrawal of the drug, a result not in full agreement with the published work. Experimental work is needed to assess further the effects of Cytosin on genitourinary tract.

Significance:

1. The promoting effect of 10% alcohol in the induction of cancer by DMBA is noteworthy. This has practical implications in the humans since a large quantity of alcohol is consumed by many people.
2. Since millions of women are put on Enovid pills, it is paramount to ascertain presence or absence of deleterious effects of the pills.
3. Cyclophosphamide is being used for a prolonged period on patients suffering from a variety of diseases. Its safety margin for the maximal therapeutic effects needs to be ascertained.

Proposed Course:

1. To study any possible cancer-promoting effects of birth control pills.
2. To study effects of chemotherapy on urinary tracts.
3. Continue to study chromosomal characteristics of established breast cell lines with special interest on the recognition of marker chromosomes by "G-bands".

Publications:

1. Bassin, R. H., Plata, E. J., Gerwin, B. I., Mattern, C. F., Haapala, D. K. and Chu, E. W.: Isolation of a continuous epitheloid cell line, HBT-3 from a Human Breast Carcinoma. Proceedings of the Society for Experimental Biology and Medicine, Vol. 141 #2, 673-680, Nov. 1972.
2. Plata, E. J., Robertson, D. D., Aoki, T., Chu, E. W. and Gerwin, B. I.: Established cultured cell lines from human breast carcinoma, JNCI, April 1973.
3. Aptekar, R. G., Atkinson, J. P., Decker, G. L., Wolff, S. M. and Chu, E.W.: Bladder toxicity with chronic oral cyclophosphamide therapy in non-malignant diseases. *Lancet* (in press)

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-854

Title: Experimental Studies on Carcinogenesis, Endocrine Features and Cytogenetic Characters

Principal Investigator: Elizabeth W. Chu, M.D.

Section: Cytopathology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: 1. To study therapeutic effects in rodents. 2. To study chromosomal expressions of spleen cells from splenectomized patients.

Approach: 1. Enovid pellets (ground Purina chow and Enovid) are given to mice as daily food. Vaginal lesions, as they arise, suspicious of being cancerous will be transplanted into other mice. 2. Suspension cells by mincing a piece of splenic tissue will be processed for chromosomal analysis by conventional Giemsa stain as well as "C"-banding for individual chromosomal recognition.

Progress: 1. The pilot study showed debatable cancerous cervical-vaginal lesions in all the mice that were fed with daily Enovid. 2. Chromosomal abnormalities were found in few of the spleens.

Significance for Cancer Research (NCP Objective 1 Approach 1,2)
Objective 5B Approach 6

Level of Effort: Professional Man-Years: 1

Serial No. NCI 517
1. Laboratory of Pathology
2. Pathological Technology
3. Bethesda, Maryland

PHS - NIH
Individual Project Report
July 1, 1972 - June 30, 1973

Part A:

Project Title: Pathological Technology Section

Principal Investigator: Miss Betty J. Sanders

Other Investigators:

Cooperating Units:

Man Years (July 1, 1972 - June 30, 1973)

Total: 15

Professional: 3

Other: 12

1. Project Description:

Stained tissue sections are the fundamental basis of all clinical and experimental studies of cancer. The Section prepares histological sections for the investigators of the National Cancer Institute. It makes available all the established routine and special stains and in addition develops and provides the current experimental methods of tissue preparation such as enzyme stains and specific histological stains.

July 1, 1972 - June 30, 1973

Number of investigators.....	110
Number of pieces of tissue.....	38,463
Number of bottles of tissue.....	7,622
Number of blocks cut.....	27,554
Number of blocks cut serially.....	579
Number of frozen blocks cut.....	39
Number of blocks recut.....	1,080
Number of slides stained H & E.....	41,685
Number of slides stained special.....	4,775
Number of unstained slides.....	2,273

Contract Work - Baker Histology Laboratory

Number of slides stained H & E.....	30,693
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2. The 186 page manual Animal Histology Procedures of the Pathological Technology Section of the National Cancer Institute was published by the Government Printing Office in September 1972. It is a compilation of the techniques used in this section. 4000 copies were printed and are available at the Government Printing Office for \$1.50 each. The manual is presently being revised to include histology techniques for lower animals in collaboration with Dr. C. Dawe and Dr. J. Harshbarger.

3. Mrs. Cervantiz Thomas is in the Upward Mobility Program working toward her B.S. degree. Her subjects have been English, Black History, Economics and Psychology. Time spent away from her job have been 10-14 hours a week. Her previous college credits have not been evaluated by Federal City College at this time and she feels she is repeating some subjects she has completed at Washington Technical Institute.

4. The Section moved from Building 8 to Building 37 in August 1972. We lost space again and have been reduced to 1370 square feet. The January 1972 move represented a loss of 2895 square feet of space.

5. The contract for processing biological microscopic slides (NCI # 71-1014) was extended in September 1972 for a period of three months. It was then combined with the contract for storage of paraffin blocks (NCI # 72-2057). The combined contract was again awarded to the Baker Histology Laboratory (Contract # 73-3863). The Baker laboratory does not produce slides of the quality necessary for most of NCI investigators but was judged by the ad hoc committee to be better than the three other bidders.

Requests for special stains and techniques are increasing. This material cannot be sent out on contract.

6. Requests from Etiology in Dr. Saffiotti's group (Drs. Rice, Ward, DeLuca and Clamon) have been made for slides because they are unable to get satisfactory slides in their own laboratory or from their contract with Microbiological Associates.

7. The section has been very active in training people from other laboratories. Consultation and training included employees of Georgetown University, Veterans Administration Hospital, Marine Biology Laboratory, Microbiological Associates, University of Arkansas (working on an HEW contract) and the Indian Medical Service Hospital, Gallup, New Mexico. Temporary Duty Assignments were arranged so the head of this section could spend 10 days each at the Arkansas and Gallup facilities for training a total of approximately 50 technicians and consulting with the pathologists in ways to improve and organize histology laboratories.

CONTRACT RESEARCH SUMMARY

Title: Maintenance of a Tissue Library and the Processing of Microscopic Slides (NCI-517)

Principal Investigator: Dr. L. B. Thomas

Name/Address

Performing

Organization: Baker Histology Laboratory, P. O. Box 48,
Great Falls, Virginia 22066

Contract Number: NIH-NCI-G-73-3863

Starting Date: December 16, 1972

Expiration Date: December 15, 1973

Goal: The contract provides histological sections of a routine nature to the investigators of the National Cancer Institute and maintains a tissue block library to file, store, and retrieve tissue embedded paraffin blocks.

Approach: The contract relieves the Pathological Technology Section of the overflow of routine preparation of histological slides of large screening experiments where quality work is not necessary. It also provides 500 square feet of space for storage of blocks.

Progress: Contract has been in effect since December 16, 1972 and as of March 21, 1973 there have been 1590 H & E slides delivered.

Significance for Cancer Research: NCP Objective 3 Approach 3.

This contract provides support for Objective 3, by supplying aid to the Pathological Technology Section in lieu of the loss of personnel and space of this Section.

Project Officer: Betty J. Sanders

Site Visit Date: Oct. 12 and 17,
1972

Program:

Technical Review Group: Dr. Gazdar, Miss Bowling, Miss Sanders, Dr. Peck,
Dr. Thomas and Dr. Dawe

Relevance Review Group: Dr. Berlin, Dr. Plough, Dr. Ketcham, Dr. Thomas,
Dr. Lutzner and Mr. Reynolds

FY73 Funds: \$104,100

1. Laboratory of Pathology
2. Surgical Pathology and
Postmortem Service
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Surgical Pathology and Postmortem Sections, together with the Cytopathology Section, Ultrastructural Pathology Section and Hematopathology Section provide complete service in anatomic pathology for the Clinical Center patients and collaborate with the research staff of all Institutes in those investigations which involve the use and study of human pathological material. The staff of the Surgical Pathology and Postmortem Sections also carries on independent and collaborative research.

Staff:

Dr. Gregory T. O'Connor, Head, Surgical Pathology Section
Dr. Ralph D. Powell, Jr., Surgical Pathology Section
Dr. Louis B. Thomas, Chief, Laboratory of Pathology and
Head, Postmortem Section
+Dr. Peter M. Banks, Resident
+Dr. Chester J. Herman, Resident
+Dr. Michael J. Lotz, Resident
+Dr. Brian R. Mullin, Resident
+Dr. Maria M. Tomaszewski, Resident
+Dr. Frank G. Witebsky, Resident
*Dr. Leon Sokoloff, Laboratory of Pathology and Histochemistry,
National Institute of Arthritis and Metabolic Diseases
*Dr. George Garrington, Deputy Director of Intramural Research,
National Institute of Dental Research
*Dr. William C. Roberts, Chief, Laboratory of Pathology,
National Heart and Lung Institute
*Dr. Marius Valsamis, Chief, Diagnostic Neuropathology, Bronx
Municipal Hospital, and Associate Professor of Pathology
(Neuropathology), Albert Einstein College of Medicine

-
- + - These physicians are full time Residents in the Laboratory of Pathology.
- * - These Associate Pathologists spend part time in the activities of the Surgical Pathology and Postmortem Sections.

Project Description:

Objectives: The objectives of the Surgical Pathology and Postmortem Sections are: (1) to provide diagnostic services in pathologic anatomy; (2) to collaborate with National Institutes of Health investigators in research involving use and study of human material; (3) to conduct a residency training program in pathologic anatomy, and (4) to carry out independent research.

1. The service functions of the Sections during the period April 1972 through March 1973 included:

(a) 224 autopsy examinations. Included in this report is a table which shows the number of autopsies performed for each Institute. The residents perform nearly all of the postmortem dissections under supervision of the staff. A gross review is held by the staff and residents each morning at 8:30. The residents review the microscopic slides of each autopsy with one of the staff before completing the autopsy protocol.

The staff assists the residents in preparing for the numerous clinical conferences in which the Sections participate.

(b) 6462 specimens were accessioned in the Surgical Pathology Section. A complete listing of these specimens, by Institute, is given in the accompanying table. These specimens are initially examined and described by a resident and their reports are checked by the staff. Associate pathologists from other laboratories or sections are frequently consulted about problems in diagnosis in surgical pathology and/or assume responsibility for handling certain tissue removed for specific research purposes.

(c) Histological preparation and special procedures:

These functions are carried out under the direction of Miss Mary C. Bowling and a complete listing of specimens processed by Institutes is given in the accompanying table.

(d) Photographic Services of the Department

A photographic record is made of a large amount of the pathological material which is handled and studied in the department. The photography is done by the staff and residents with the technical assistance of Mr. Kenzie Edwards who also maintains the equipment and carries out much of the photographic processing. Gross photographs and photomicrographs of the pathologic material are provided to the clinical staff on request, are used extensively for conferences and seminars and many are prepared for publication.

During the past year Mr. Edwards took 2241 color negatives, 2220 black and white negatives and 207 rolls of 35 mm. color film. Prints made outside totaled 8706 and prints made here totaled 66. Time taking photographs: 622 hours; time processing: 375 hours.

REPORT OF POSTMORTEM PATHOLOGY SECTION

APRIL 1, 1972 through March 31, 1973

INSTITUTE AND TYPE OF PATIENT

	NCI	NHLI	NIAND	NIAID	NINDS	NIMH	NIDR	NEI	NICHD	REF.	TOTAL
<u>Autopsies performed</u>	117	24	11	1	7	0	0	0	2	0	162
<u>Autopsies submitted</u>	38	12	1	1	2	0	0	0	0	8	62
<u>Total autopsies</u>	155	36	12	2	9	0	0	0	2	8	224
<u>Blocks of tissue cut</u>	9733	1546	751	120	438	0	0	0	139	22	12749
<u>Blocks of tissue recut</u>	1047	123	104	13	101	0	0	0	21	3	1412
<u>Blocks serialled</u>	5	0	0	0	0	0	0	0	0	0	5
<u>Total blocks cut</u>	10785	1669	855	133	539	0	0	0	160	25	14166
<u>Slides made</u>	13828	2255	1098	173	813	0	0	0	207	40	18414
<u>Slides H&E stained</u>	10252	1562	785	122	500	0	0	0	150	148	13519
<u>Slides special stained</u>	1758	251	206	41	175	0	0	0	31	15	2477
<u>Total slides stained</u>	12010	1813	991	163	675	0	0	0	181	163	15996
<u>Frozen sections</u>	28	8	0	0	9	0	0	0	0	0	45

CONTRACT: 26 autopsies sent (1661 pieces of tissue)

1660 H&E stained slides returned

REPORT OF SURGICAL PATHOLOGY SECTION

APRIL 1972 through MARCH 31, 1973

INSTITUTE AND TYPE OF PATIENT

	NCI	NHLI	NIAMD	NIAMD	NINDS	NIMH	NIDR	NEI	NICHD	REF.	RES.	TOTAL
Specimens accessioned	4226	372	348	199	87	7	685	12	46	390	90	6462
Blocks of tissue cut	9338	564	736	373	293	15	31	35	110	468	122	12085
Blocks of tissue recut	580	59	82	67	33	0	16	4	22	33	0	896
Total blocks cut	9918	623	818	440	326	15	47	39	132	501	122	12981
Slides made	29825	1782	3574	1668	857	35	164	97	525	3442	244	42213
Slides stained H&E	17916	832	1989	918	462	18	558	49	227	2054	143	25166
Slides stained special	4557	228	351	313	146	1	32	6	171	339	0	6144
Total slides stained	22473	1060	2340	1231	608	19	590	55	398	2393	143	31310
Frozen Sections	346	31	80	10	29	2	1	0	14	1	0	514

Total Surgical and Postmortem Pathology Section Report:

Number of blocks cut-----26961
 Number of slides made-----60617
 Number of slides stained H&E-----38620
 Number of slides stained special-----8621

A large proportion of the photomicrography is done under the direction of Mr. Ralph Isenberg who provides professional assistance and facilities for the entire staff of the Laboratory of Pathology.

2. The collaborative activities of the staff and residents in the Surgical Pathology and Postmortem Sections are reflected in the summary of research activities listed below:

(a) Distribution of human organs and tissues

At present there are approximately 50 scientists in the various laboratories at the National Institutes of Health who have requested particular tissues from surgical and postmortem services. On many occasions it is possible to furnish these investigators with fresh human material. A list of these investigators is kept by the secretarial staff and telephone calls are made to the various investigators whenever surgical or postmortem tissues are available.

(b) Conferences:

The staff and residents take an active part in numerous Clinical Center and Institute conferences. In addition, there are several conferences regularly held in the department, including a Surgical Pathology Conference, Joint Pathologists Conference, Neuropathology Conference, Staging Conference, and Gross Autopsy Conference.

A. Dr. Gregory T. O'Connor:

1. Studies of human and Simian herpes viruses. The oncogenicity and virus cell relationships of these viruses are being investigated in vitro and in vivo. In collaboration with Dr. Alan Rabson, and Dr. Nubia Munoz, IARC.
2. Electron microscopy study of selected tumor and tumor cell lines. These studies involve screening for viruses and a search for specific morphological changes related to the presence of viral genome.
3. Electron microscopy of selected specimens from the surgical pathology service. The objective is to improve the accuracy of diagnoses in surgical pathology by defining ultrastructural characteristics of specific tissues.
4. Studies in American patients with Burkitt's tumor. In collaboration with VLL, DCCP.
5. Epidemiological studies of Hodgkin's disease. In collaboration with Biometry Branch and VLL, DCCP.

6. Other activities:

(a) Chairman, Commission on Epidemiology of UICC.

Responsibility includes coordination of the research program of three committees and the planning of and participation in their meetings. I am actively participating in the investigations in progress by two of these committees, i.e. Childhood Cancer and Hodgkin's Disease.

(b) Member, Executive Committee, UICC.

(c) Chairman, U.S. National Committee, UICC.

(d) Member, Breast Cancer Task Force, NCI.
Chairman, Committee on Epidemiology.(e) Member, Committee on Geographic Pathology,
International Academy of Pathology.(f) Head, Collaborating Center for International Reference Center
on Classification of Neoplasms of the Hematopoietic System.(g) Member, N.Y. State Cancer Control Bureau
Scientific Advisory Group.(h) Chairman, Ad hoc committee on International Nomenclature,
Coding and End Results, AACI.

(i) Consultant, International Agency for Research on Cancer.

(j) Consultant, Sero-epidemiology Contract Review Committee, NCI.

(k) Consultant - USA Committee of International Societies of Pathology,
NRC and Project Officer for Contract to support WHO International Reference Centers.

(l) Member, WHO Expert Advisory Panel on Cancer.

(m) Special Assistant to Director for International Affairs, NCI.

Publications:

1. Levine, P.H., O'Connor, G.T., and Berard, C.W.: Antibodies to Epstein-Barr virus (EBV) in American patients with Burkitt's lymphoma. Cancer 30: 610-615, 1972.
2. Stevens, D.A., O'Connor, G.T., Levine, P.H., and Rosen, R.B.: Acute leukemia with "Burkitt's lymphoma cells" and Burkitt's lymphoma. Ann. Intern. Med. 76: 967-973, 1972.

3. Krueger, G., and O'Connor, G.T.: Epidemiologic and immunologic considerations on the pathogenesis of Burkitt's tumor. In Grundmann, E. and Tulinius, H. (Ed.): Recent Results in Cancer Research. Berlin, Springer-Verlag, 1972, Vol. 39, pp. 211-224.
4. Elgort, D.A., Abelev, G.I., and O'Connor, G.T.: Dependence of the specificity of the serologic test for primary liver cancer in different areas of the world on sensitivity of the method used for detecting alpha-fetoprotein. Int. J. Cancer 10: 331-337, 1972.
5. Levine, P.H., Sandler, S.G., Komp, D.M., O'Connor, G.T., and O'Connor, D.M.: Simultaneous occurrence of "American Burkitt's lymphoma" in neighbors. N. Engl. J. Med. 288: 562-563, 1973.
6. O'Connor, G.T., Correa, P., Christine, B., Axtell, L., and Myers, M.: Hodgkin's disease in Connecticut: Histology and age distribution. J. Natl. Cancer Inst. Monograph. In Press.
7. O'Connor, G.T., and Templeton, A.C.: Tumours of the respiratory tract. Recent Results in Cancer Research. Vol. 41. In Press.
8. Krueger, G.R.F., O'Connor, G.T., Sussman, E., and Tralka, T.S.: Ultrastructure and tissue culture studies of a thymic lymphoma induced by persistent antigenic stimulation and immunosuppression. Zeitschrift fur Krebsforschung. In Press.
9. Correa, P., O'Connor, G.T., Berard, C.W., Axtell, L.M., and Myers, M.H.: International comparability and reproducibility in histological subclassification of Hodgkin's disease. J. Natl. Cancer Inst. In Press.
10. Correa, P., and O'Connor, G.T.: Geographic pathology of lymphoreticular tumors. Summary of survey from the Geographic Pathology Committee of the UICC. J. Natl. Cancer Inst. In Press.

B. Dr. Ralph D. Powell, Jr.:

1. A study of the natural history of bronchogenic carcinoma and the effect of therapy on survival. (Patients are randomly assigned to radiotherapy alone or radiotherapy with adjuvant chemotherapy). In collaboration with Drs. R.E. Johnson and K.B. Lamoureux, Radiation Branch, NCI.
2. Pneumocystis carinii pneumonia in cancer patients following immunosuppressive chemotherapy: Results of pentamidine isethionate treatment (A clinical-pathological study of 80 NIH patients). In collaboration with Drs. G.W. Geelhoed, V.T. DeVita, NCI, and Dr. J.L. Doppman, DR, CC.

3. The uterine cervix as the presenting site of metastatic carcinoma. (A report of two cases with review of the literature). In collaboration with Dr. P. Chretien, NCI, and Dr. S.H. Bennett, Johns Hopkins.
4. Nasal biopsy in Sjogren's disease. (A morphologic study of the changes in the nasal biopsies from 12 patients with Sjogren's disease with comparison to changes at other sites, including lip biopsies). In collaboration with Dr. R.I. Henkin, NHLI.
5. Nasal biopsy in disorders of taste and smell. (A study of nasal biopsies from approximately 300 patients, including many with disorders of taste and smell). In collaboration with Dr. R.I. Henkin, NHLI.
6. Pulmonary diseases occurring in an autopsy population of Cairo, Egypt, UAR. (Lung tissue from a year of consecutive autopsy cases is being reviewed both in Cairo and in Bethesda). In collaboration with Dr. A.W. Cheever, Cairo.
7. Progressive airway obstruction occurring in a patient with Sjogren's disease (report of a case in which lung biopsy revealed chronic stenosing bronchiolitis). In collaboration with Drs. C.A. Hall and H.H. Newball, NHLI.
8. The response of pulmonary alveolar ducts to bronchoconstrictive agents. (In laboratory animals and in surgically-excised human lung, the effect of bradykinin and allied agents is being studied physiologically and anatomically). In collaboration with Dr. H.H. Newball, NHLI.
9. Active tumor immunotherapy with BCG. In collaboration with Dr. E. Rosenberg, Papanicolaou Cancer Research Institute. Submitted to Southern Med. J.
10. Sexual maturation in bulbectomized female rats. In collaboration with Dr. R.I. Henkin, NHLI, Dr. N. Sato, formerly NHLI, and Dr. E.W. Haller, University of Minnesota, Duluth, Minnesota. Submitted to J. Reprod. and Fertility.
11. Testicular steroidogenesis and morphology in zinc deficiency. In collaboration with Dr. N.K. Prakash, formerly NHLI, and Dr. R.I. Henkin, NHLI. Submitted to Endocrinology.
12. Ovarian morphology in women with anosmia and hypogonadotrophic hypogonadism. In collaboration with Dr. R.L. Goldenberg, Yale University Medical School, Dr. S.W. Rosen, NIAMD, Dr. J.R. Marshall, formerly NICHD, and Dr. G.T. Ross, NICHD. Submitted to Endocrinology.

Publication:

1. Cook, A.J., Weinstein, M., and Powell, R.D.: Diffuse amyloidosis of the tracheobronchial tree. Radiology. In Press.

Other Activities:

1. Participant, Collaborating Center, International Reference Center for Classification of Uterine and Placental Tumors.

C. Dr. Peter M. Banks:

1. Review of melanoma cases in collaboration with Dr. Alan Rabson, Dr. G.W. Geelhoed, and Dr. A.S. Ketcham, NCI.
2. Case Report: Histiocytic medullary reticulosis, in collaboration with Drs. Douglas Hurley and Sheldon Wolff, NIAID.
3. Case Report: Light and electron microscopic findings in a case of acute myocardial degeneration after high dose cyclophosphamide therapy. In collaboration with Dr. L. Max Buja, NHLI.
4. Review of autopsy findings among 19 cases of American Burkitt's lymphoma. In collaboration with Dr. Costan Berard, NCI.

D. Dr. Chester J. Herman:

Since research projects are associated with cytopathology, they are listed under Serial No. 589, Cytopathology Service.

E. Dr. Michael J. Lotz:

1. Experimental study of implantation of tissue culture osteosarcoma into guinea pig femur. In collaboration with Dr. R. Lewis of Surgery Branch, NCI.

Publications:

1. Lewis, R., and Lotz, M.J.: Extra-osseous osteosarcoma. Am. Surgeon. In Press.
2. Lewis, R., and Lotz, M.J.: Medullary extension of osteosarcoma. Cancer. In Press.

F. Dr. Maria M. Tomaszewski:

1. Review and adaptation of the Breast Tumor Service (BTS) Protocol 10-6(5). In collaboration with Dr. G.T. O'Connor.

G. Dr. Frank G. Witebsky:

1. The effect of sensory nerve transection on sensory end organs in the skin. In collaboration with Dr. R.D. Powell, Jr., Dr. A.L. Dellon, and Dr. P. Chretien, NCI.

H. Mary C. Bowling:

1. Bowling, M.C., Smith, I.M., and Wescott, S.L.: A rapid staining procedure for pneumocystis carinii. Submitted to Am. J. Med. Technol.

Serial No. NCI 555

1. Laboratory of Pathology
2. Ultrastructural Pathology
Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cytochemical and Immunological Studies of Membrane Structures
Associated with Arbovirus Replication

Previous Serial Number: Same

Principal Investigator: Philip M. Grimley, M.D.

Other Investigators: I.K. Berezsky, J.G. Levin, and N. Young

Cooperating Units: Channing Laboratory, Boston City Hospital

Man Years (July 1, 1972 - June 30, 1973)

Total: 1

Professional: 1

Other:

Project Description:

Objectives: To examine the role of membranes in arbovirus replication.

Major Findings: In tissue culture and experimental animals ultra-structurally unique cytoplasmic membranes have been identified in cells infected by group A arboviruses. These membranes, designated type 1 cytopathic vacuoles (CPV-1), are sites of virus RNA replication. This was demonstrated by high resolution autoradiography and cell fractionation.

As infection progresses, arbovirus nucleoids accumulate in the cytoplasm and mature by budding through other host cell membranes, either cytoplasmic vacuoles or endoplasmic cisternae, or at the cell surface. Our studies have demonstrated both pathways of virus envelopment in vivo and in vitro.

Intracytoplasmic membranous structures of a unique type were associated with the replication of three group A arboviruses. The structures, referred to as type 1 cytopathic vacuoles (CPV-1), were membrane-limited and characteristically lined by regular membranous spherules measuring 50 nm in diameter. Detection of CPV-1 coincided with the time of rapid virus growth in several different cell types and preceded the accumulation of virus nucleocapsids. Counts of CPV-1 remained in a similar range even when the multiplicity of infection was varied over 100-fold. Inhibition of cellular RNA and protein synthesis by actinomycin D did not decrease the counts of CPV-1; however, biogenesis of CPV-1 was limited when viral replication was

inhibited by inhibitors of viral RNA synthesis (guanidine) or of viral protein synthesis (cycloheximide). It was concluded that formation of CPV-1 resulted from a virus-specified modification of pre-existing host cell structures. Cytochemistry suggested that Golgi cytomembranes might be involved.

When cytoplasmic extracts of cells infected with an arbovirus were subjected to isopycnic centrifugation in discontinuous sucrose gradients, seven distinct bands were usually formed. The four upper bands contained predominantly smooth membranes and the lowest band was enriched in rough endoplasmic reticulum. One fraction (fraction 5), banding at a density of 1.16 gm/cm^3 , was found to be heavily enriched in pulse-labeled ribonucleic acid (RNA), viral RNA polymerase, and viral RNA forms associated with RNA replication. This fraction evidently contained a membrane-associated viral replication complex. Fraction 5 was also consistently enriched with unique membranous structures previously observed in intact cells as type 1 cytopathic vacuoles (CPV-1). When the CPV-1 in fraction 5 were isolated from cells briefly incubated with ^3H -uridine and ^3H -adenosine prior to cell disruption, a large proportion were found to be labeled by high resolution autoradiography. Thus, ultrastructural, biochemical and biological evidence were all consistent with the interpretation that CPV-1 membranes represent a significant element of the arboviral replication complex.

Significance: Identification and study of membranous structures associated with arbovirus replication contributes basic information to virology and molecular biology. The group A arboviruses include several important pathogens and studies in progress are providing basic information concerning the pathogenesis of infections.

Proposed Course: The significance and fate of virus replicative membranes will be examined further utilizing cytochemistry and conjugated antibodies.

Publications:

1. Grimley, P.M., Levin, J.G., Berezesky, I.K., and Friedman, R.M.: Specific membranous structures associated with the replication of group A arboviruses. J. Virol. 10: 492-503, 1972
2. Friedman, R.M., Levin, J.G., Grimley, P.M., and Berezesky, I.K.: Membrane-associated replication complex in arbovirus infection. J. Virol. 10: 504-515, 1972

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-555

Title: Cytochemical and Immunological Studies of Membrane Structures
Associated with Arbovirus Replication

Principal Investigator: Philip M. Grimley, M.D.

Section: Ultrastructural Pathology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To examine virus-induced modifications of host cell membranes.

Approach: Specific membranous structures which appear in the cytoplasm of cells infected by group A arboviruses will be examined by cytochemical and immunological techniques coupled with electron microscopy.

Progress: Specific membranous structures were isolated in sucrose gradients and their association with a viral replication complex was demonstrated by combined biochemical, autoradiographic and ultrastructural techniques.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Specific effect of virus infection on host cell membranes has been demonstrated. Similar mobilization of host cell membranes for replication of tumor viruses could be an important factor in malignant transformation.

Level of Effort: Professional Man-Years: 1

Serial No. NCI 555a

1. Laboratory of Pathology
2. Ultrastructural Pathology
Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Ultrastructural and Biological Studies of Effects of Rifamycin
Derivatives on Poxvirus Infections

Previous Serial Number: Same

Principal Investigator: Philip M. Grimley, M.D.

Other Investigators: B. Moss, E. Rosenblum, and S.J. Mims

Cooperating Units: Laboratory of Biology Viruses, NIAID

Man Years (July 1, 1972 - June 30, 1973)

Total: 1 1/2

Professional: 1 1/2

Other:

Project Description:

Objectives: 1. To examine the effects of rifamycin derivatives on molecular events in poxvirus assembly. 2. To examine the structure and formation of poxvirus membranes. 3. To study events in poxvirus morphogenesis.

Major Findings: The antibiotic rifampicin blocks poxvirus assembly. Morphogenesis is interrupted at a stage prior to formation of virus envelopes. Viral membrane which accumulate in the presence of rifampicin lack a normal outer coating. When the drug is removed, the morphologic effect is rapidly reversed. The specific effect of rifampicin on the formation of the vaccinia viral envelope was reversed within 10 minutes even in the presence of NaF or inhibitors of protein or nucleic acid synthesis. Further maturation of some virus particles, which were characterized by their ultrastructure and isolated by sucrose gradient sedimentation, proceeded in the presence of inhibitors of protein synthesis. In contrast, addition of NaF or actinomycin D almost completely blocked the development of mature virus particles. Rifampicin acted specifically at the stage of envelope formation and did not prevent later steps in maturation.

A rifampicin-resistant vaccinia virus mutant was isolated from wild-type virus stocks. Growth of the mutant was inhibited only slightly at concentrations of rifampicin which completely prevented the growth of drug-sensitive virus, and at no concentration tested was there a detectable effect on viral envelope formation. The mutant virus was capable of rescuing wild-type during

mixed infection in the presence of rifampicin; after simultaneous infection the ratio of genomes in the progeny reflected that of the original inoculum. Superinfection experiments demonstrated that heat-inactivated mutant was capable of efficiently rescuing wild-type virus and that extensive replication of the mutant genome was not required for this. Characteristic rifampicin blocked viral forms, normal maturing forms, and intermediate structures were seen in the same ultrathin cell sections after simultaneous infection with low multiplicities of mutant and wild-type virus. In cells simultaneously infected with high multiplicities of mutant and wild-type virus, rifampicin had no detectable effect on viral envelope formation.

Membrane-limited structures, resembling virus envelope precursors previously shown to form during the interruption of poxvirus assembly by rifampin, were also observed by electron microscopy in vaccinia-infected HeLa cells treated with a series of rifamycin derivatives. The active compounds N-demethyl rifampin, AF/DMI, and 3-formyl rifamycin SV lacked, respectively, a methyl group, the piperazine ring, and the hydrazone portion of rifampin. A vaccinia mutant selected only for resistance to rifampin was also resistant to the effect on morphogenesis produced by all of the rifamycin derivatives. We concluded that this antiviral effect was specific and was a property associated with the macrocyclic ring rather than the hydrazone containing side chain of rifampin. In addition to their effects on vaccinia morphogenesis, 3-formyl rifamycin SV and AF/DMI had unusual cytotoxic effects, including swelling of mitochondria.

Utilizing a selected series of rifamycin derivatives with modified piperazinylinomethyl side chains, it was determined that minor lengthening of the side chain decreased specific antiviral activity as determined by infectivity and electron microscopy. An amino-substituted analog of rifampicin, however, was found to have increased specific activity.

Significance: The action of rifamycin antibiotics on poxvirus assembly is unique and provides a novel approach to studies of the macromolecular organization of viruses and of virus membrane specialization. Analysis of poxvirus membrane development may lead to further understanding of membrane bioassembly processes. Studies of the rifamycin antibiotics are of broad fundamental importance in developing therapeutically useful antiviral agents.

Proposed Course: Events in the bioassembly of poxvirus envelopes will be examined utilizing rifampicin as a probe. The technique of freeze-fracture can be employed to examine these membranes as a model of maturation.

Publications:

1. Moss, B., Rosenblum, E.N., Grimley, P.M., and Mims, S.J.: Rifamycins: Modulation of specific anti-poxviral activity by small substitutions on the piperazinylinomethyl side chain. Antimicrob. Agents Chemother. 2: 181-185, 1972

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-555a

Title: Ultrastructural and Biological Studies of Effects of Rifamycin Derivatives on Poxvirus Infections

Principal Investigator: Philip M. Grimley, M.D.

Section: Ultrastructural Pathology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: 1. To examine the effects of rifamycin derivatives on molecular events in poxvirus assembly. 2. To examine the structure and formation of poxvirus membranes. 3. To study events in poxvirus morphogenesis.

Approach: A specific antiviral effect of a selected series of rifamycin derivatives was investigated by means of infectivity assay and quantitative electron microscopy.

Progress: We concluded that the specific antiviral property of rifampicin was associated with the macrocyclic ring rather than with the hydrazone containing side-chain. Minor lengthening or shortening of the side-chain decreased specific antiviral activity; however, an amino-substituted analog was found to possess increased specific activity.

Significance for Cancer Research (NCP Objective 6 Approach 2)

The action of rifamycin antibiotics on poxvirus assembly is unique and provides a novel approach to studies of the macromolecular organization of viruses and of virus membrane specialization. Analysis of poxvirus membrane development may lead to further understanding of membrane bioassembly processes. Studies of the rifamycin antibiotics are of broad fundamental importance in developing therapeutically useful antiviral agents.

Level of Effort: Professional Man-Years: 1 1/2

Serial No. NCI 577

1. Laboratory of Pathology
2. Ultrastructural Pathology
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ultrastructural and Biologic Studies of Tubuloreticular Structures in Neoplastic and Virus-infected Cells

Previous Serial Number: Same

Principal Investigator: Philip M. Grimley, M.D.

Other Investigators: J.L. Decker, Z. Schaff, D.W. Barry, and H.J. Michelitch

Cooperating Units: Arthritis and Rheumatism Branch, NIAMD

Man Years (July 1, 1972 - June 30, 1973)

Total: 1
Professional: 1
Other:

Project Description:

Objectives: To evaluate the significance of tubuloreticular structures in neoplastic cells, virus-infected cells, and cells of patients with connective tissue diseases.

Major Findings: Tubuloreticular structures were found in the circulating lymphoid cells of patients with systemic lupus erythematosus and in some related diseases of connective tissue. They resembled structures previously described in glomerular endothelium of these patients. Tubules measured 220-240 Angstroms and were located in the granular endoplasmic reticulum.

The specificity of the lymphocyte findings was established in a prospective blind study including healthy controls and patients with infectious mononucleosis or rheumatoid arthritis. The tubuloreticular structures were found only in lymphocytes of patients with lupus erythematosus, Sjögren's syndrome, polymyositis and scleroderma.

Significance: The biologic nature of tubuloreticular structures and factors leading to their pathogenesis remains to be ascertained. The selectivity of cell involvement in several specific disease processes, including cancer, suggests a broad biological significance.

Proposed Course: The nature of tubuloreticular structures in connective tissue disease and in neoplastic cells will be investigated by a variety of techniques including cytochemistry and immuno-electron microscopy. Human lymphoid cell cultures will be utilized as an experimental model.

Publications:

1. Grimley, P.M.: Arthritis Rounds--Discussion of tubular structures in endothelium in palindromic rheumatism. Arthritis Rheum. 15: 555-556, 1972

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-577

Title: Ultrastructural and Biologic Studies of Tubuloreticular Structures in Neoplastic and Virus-infected Cells

Principal Investigator: Philip M. Grimley, M.D.

Section: Ultrastructural Pathology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To evaluate the significance of tubuloreticular structures in neoplastic cells, virus-infected cells, and cells of patients with connective tissue diseases.

Approach: Tubuloreticular structures were found in the circulating lymphoid cells of patients with systemic lupus erythematosus and in some related diseases of connective tissue. They resembled structures previously described in glomerular endothelium of these patients. Tubules measured 220-240 Angstroms and were located in the granular endoplasmic reticulum.

Progress: The specificity of the lymphocyte findings was established in a prospective blind study including healthy controls and patients with infectious mononucleosis or rheumatoid arthritis. The tubuloreticular structures were found only in lymphocytes of patients with lupus erythematosus, Sjögren's syndrome, polymyositis and scleroderma.

Significance for Cancer Research (NCP Objective 3 Approach 3)

The biologic nature of tubuloreticular structures and factors leading to their pathogenesis remains to be ascertained. The selectivity of cell involvement in several specific disease processes, including cancer, suggests a broad biological significance.

Level of Effort: Professional Man-Years: 1

Serial No. NCI 582

1. Laboratory of Pathology
2. Ultrastructural Pathology
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Pathogenesis of Chronic Mouse Cytomegalovirus Infection in Lymphoid Organs and Effect of Chronic Infection on Development of Lymphoma

Previous Serial Number: Same

Principal Investigators: Donald E. Henson, M.D.; Margery Sullivan; and Duran Harris

Other Investigators: A.J. Strano

Cooperating Units: Armed Forces Institute of Pathology

Man Years (July 1, 1972 - June 30, 1973)

Total: 1
Professional:
Other:

Project Description:

Objectives: Study the mechanisms of latent murine cytomegalovirus infection in the lymphoid tissue of mice chronically infected with this virus and determine effect of chronic infection on lymphoma development.

Methods Employed: Tissue culture, immunofluorescence, electron microscopy.

Significance: The mouse cytomegalovirus causes chronic infection in the submaxillary glands, lymph nodes, and spleens of many strains of mice. Infection in the lymph nodes and spleen probably occurs in lymphocytes. The murine cytomegalovirus belongs to the herpesvirus group of animal viruses, along with the Epstein-Barr virus and Herpesvirus saimiri. Consequently, a study of murine cytomegalovirus infections in lymphocytes may help solve some of the problems regarding other herpesvirus infections of lymphocytes and relationship of chronic infection to lymphomas.

Proposed Course: Investigate 1) latent cytomegalovirus infection in lymphoid tissue and 2) effect of chronic infection on chemical induced lymphomas in mice.

Publications:

1. Henson, D.E., and Strano, A.J.: Mouse cytomegalovirus: Necrosis of infected and morphologically normal submaxillary gland acinar cells during termination of chronic infection. Am. J. Path. 68: 183-202, 1972
2. Henson, D.E., Strano, A.J., Slotnik, M., and Goodheart, C.: Mouse cytomegalovirus: Isolation from lymphoid tissue of chronically infected mice. Proc. Soc. Exp. Biol. Med. 140: 802-806, 1972
3. Henson, D.E., Siegel, S.E., Fuccillo, D.A., Matthew, E., and Levine, A.S.: Cytomegalovirus infections during acute childhood leukemia. J. Infect. Dis. 126: 469-481, 1972

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 582

Title: Pathogenesis of Chronic Mouse Cytomegalovirus Infection in Lymphoid Organs and Effect of Chronic Infection on Subsequent Development of Lymphoma

Principal Investigator: Donald E. Henson, M.D.

Section: Ultrastructural Pathology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: Study the mechanism of latent murine cytomegalovirus infection and the effect of chronic infection on the incidence of lymphoma development in mice.

Approach: Involves an electron microscopic, tissue culture, and immunofluorescent study of chronically infected mice and factors such as immunosuppression that effect the course of chronic infection. In addition chronically infected mice are being treated with chemical carcinogens and the incidence of tumors will be compared to non-infected mice treated with the same carcinogens.

Progress: Mouse cytomegalovirus causes chronic infection in the salivary glands and leucocytes. Furthermore, evidence indicates that cells from chemically induced lymphomas are chronically infected with the mouse cytomegalovirus. Results demonstrating possible enhancing effect of the mouse cytomegalovirus on incidence of chemically induced tumors are not yet available.

Significance for Cancer Research (NCP Objective 1 Approach 4)

Level of Effort: Professional Man-Years: 1

Serial No. NCI 583

1. Laboratory of Pathology
2. Ultrastructural Pathology
Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunological, Biological, and Ultrastructural Studies of
Leucocytes Infected with Herpes-type Viruses

Previous Serial Number: 863c

Principal Investigators: Donald E. Henson, M.D.; Philip M. Grimley, M.D.;
Margery Sullivan; and Duran Harris

Other Investigators: None

Cooperating Units: None

Man Years (July 1, 1972 - June 30, 1973)

Total: 1

Professional:

Other:

Project Description:

Objectives: 1) Develop immunologic and ultrastructural techniques for examination of surface antigens and receptor sites on human leucocytes and on tumor cells. 2) Study effects of overt and covert infections of herpes-type viruses on distribution and types of surface receptors on leucocytes and other cells.

Major Findings: 1) Hybrid antibody technique was used to label immunoglobulin E surface receptors on human basophils. 2) Direct antibody labeling technique has been developed to localize herpesvirus antigen in cultured lymphocytes.

Significance: Much evidence indicates that the lymphoid system is a target organ for herpes-type viruses. Development of immunologic and ultrastructural techniques will aid in our understanding of virus expression in leucocytes in clinical conditions such as lymphomas or lupus erythematosus.

Proposed Course: Hybrid antibody method of immunolabeling for electron microscopy is nearing final stage of development in our laboratory. After development, system will be applied to tumor antigens and to virus infected cells.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-583

Title: Immunological, Biological, and Ultrastructural Studies of Leucocytes Infected with Herpes-type Viruses

Principal Investigator: Donald E. Henson, M.D.

Section: Ultrastructural Pathology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: Study effects of virus infection on cell surface antigens.

Approach: Study the effect of overt and covert virus infections on the distribution of normal surface antigens on cells by immunolabeling methods for electron microscopy.

Progress: Hybrid antibody technique is nearing final stages of development in our laboratory. Some studies have already been done showing the distribution of antigenic sites on cells infected with herpes simplex virus. It is hoped to eventually apply these methods to tumor antigens.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Level of Effort: Professional Man-Years: 1

Serial No. NCI 585

1. Laboratory of Pathology
2. Ultrastructural Pathology
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Histological Evolution of In Situ Carcinoma in the Vagina

Previous Serial Number: Same

Principal Investigators: Donald E. Henson, M.D.; Peyton T. Taylor, M.D.;
and Glenn W. Geelhoed, M.D.

Other Investigators: None

Cooperating Units: Surgery Branch

Man Years (July 1, 1972 - June 30, 1973)

Total: 1/4

Professional: 1/4

Other:

Project Description:

Objectives: Clinical-pathological study of women with multicentric squamous cell cancers in the ectogenital epithelium.

Major Findings: Histological studies made on women with sequential vaginal biopsies have demonstrated progression from severe epithelial atypia, through dysplasia, to carcinoma in situ. As carcinoma of the cervix, in situ vaginal tumors have an invasive potential. Lesions seem to enlarge by intra-epithelial invasion.

Significance: Many women with cancer of the uterine cervix subsequently develop epithelial cancers in the vagina and/or vulva. These multiple cancers have been considered to arise in an epithelial region predisposed to malignancy. This region has a common cloacal origin embryologically.

Proposed Course: Project is still in effect. Patients will be added to the study as they are diagnosed.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-585

Title: Histological Evolution of In Situ Carcinoma of the Vagina

Principal Investigator: Donald E. Henson, M.D.; Peyton T. Taylor, M.D.;
Section: Ultrastructural Pathology and Glenn W. Geelhoed, M.D.

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: Determine if vaginal in situ carcinoma represents a diffuse field change in the ectogenital epithelium and the invasive potential of this lesion.

Approach: Histological study of vaginal biopsy material obtained from women who are being followed by the Surgery Branch for in situ carcinoma of the vagina.

Progress: This is an ongoing study. Patients are being added to the project as they are diagnosed in the Clinic.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Level of Effort: Professional Man-Years: 1/4

Serial No. NCI 863a
1. Laboratory of Pathology
2. Ultrastructural Pathology
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ultrastructural Studies of Human Cytology Specimens

Previous Serial Number: Same

Principal Investigator: Philip M. Grimley, M.D.

Other Investigators: E.W. Chu, I.K. Berezsky, H.J. Michelitch, and
T.A. Wood, Jr.

Cooperating Units: None

Man Years (July 1, 1972 - June 30, 1973)

Total: 1/4
Professional: 1/4
Other:

Project Description:

Objectives: To compare the ultrastructure of normal and neoplastic cells in samples of body fluids.

Major Findings: Preliminary correlation of electron micrographs with cytology specimens suggests that histiocytes and mesothelial cells in pleural fluids may be distinguished from neoplastic cells by ultrastructural features.

Significance: Results contribute basic information regarding tumor cell structure and may assist in cytodiagnosis.

Proposed Course: To continue examination of cell preparations by transmission and scanning electron microscopy.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-863a

Title: Ultrastructural Studies of Human Cytology Specimens

Principal Investigator: Philip M. Grimley, M.D.

Section: Ultrastructural Pathology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To compare the ultrastructure of normal and neoplastic cells in samples of body fluids.

Approach: Examination of cell preparations by transmission and scanning electron microscopy.

Progress: Preliminary correlation of electron micrographs with cytology specimens suggests that histiocytes and mesothelial cells in pleural fluids may be distinguished from neoplastic cells by ultrastructural features.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Results contribute basic information regarding tumor cell structure and may assist in cytodiagnosis.

Level of Effort: Professional Man-Years: 1/4

Serial No. NCI 531

1. Laboratory of Pathology
2. Viral Oncology and Molecular Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: In Vitro Studies of Human Malignant Tumors

Previous Serial Number: Same

Principal Investigator: Alan S. Rabson, M.D.

Other Investigators: N. Munoz, G.T. O'Connor, E.W. Chu, D. Albert (Yale University), and S. Rosen (NIAMD)

Cooperating Units: None

Man Years (July 1, 1972 - June 30, 1973)

Total: 1

Professional: 2/3

Other: 1/3

Project Description:

Objectives: To study biological properties of human lymphoma, sarcoma, carcinoma and retinoblastoma cells in vitro and to attempt to isolate oncogenic viruses from these cells.

Methods Employed: Standard tissue culture, virologic and pathologic techniques are used.

Major Findings: We have continued to isolate cell lines from human tumors and to attempt to isolate and identify oncogenic viruses from these cell lines. Thus far no human oncogenic viruses have been isolated.

Studies have continued with the human cell line isolated from the human bronchogenic carcinoma (Chago line). The cells continue to produce human chorionic gonadotropin (hCG) in culture after 2 years in culture. Although they produce small amounts of hCG, they produce large amounts of the alpha subunit of hCG (as much as 2000 nanograms per ml of culture fluid). Clonal lines have been derived in collaboration with Dr. A. Tashjian at Harvard Medical School and some produce large amounts of the alpha subunit while others produce large amounts of the beta subunit. These findings are analogous to the unbalanced synthesis of immunoglobulin chains in some neoplastic plasma cells.

We have continued our studies of human retinoblastomas in culture and in collaboration with Dr. D. Albert (Dept. Ophthalmology, Yale University) we have established two continuous cell lines of human retinoblastoma cells. These cells grow in suspension and still form rosettes but no viral particles have been detected by electron microscopy and no viruses have been isolated from them in culture. We have also continued our studies of pigment epithelium in culture and succeeded in transforming pigment epithelium in vitro with oncogenic viruses and producing tumors in animals with the transformed cells.

We have continued our studies of human carcinoma of the cervix and our attempts to demonstrate viral antigens in cell lines derived from human cervical cancer but none have been detected thus far. We have identified sera of patients with cervical cancer which contain high titers of antibody against Herpesvirus hominis, type 2, antigens and we are using these sera in our studies of human cervical cancer.

Significance: The importance of the search for possible viral etiology of human malignant tumors is evident. In addition, any information about the biological properties of human tumor cells may be of importance in our understanding of the lymphoma problem.

Proposed Course: Attempts to isolate viruses from human malignant tumors using a variety of techniques which have been successful in the isolation of oncogenic viruses in animal systems will be continued. Studies of factors involved in human chorionic gonadotropin production in vitro in lung cancer cells will be initiated.

Publications:

1. Rabson, A.S., Rosen, S.W., Tashjian, Jr., A.H., and Weintraub, B.D.: Production of human chorionic gonadotropin in vitro by a cell line derived from a carcinoma of the lung. J. Natl. Cancer Inst. (in press)
2. Tashjian, Jr., A.H., Weintraub, B.D., Barowsky, N.J., Rabson, A.S., and Rosen, S.W.: Subunits of human chorionic gonadotropin: Unbalanced synthesis and secretion by clonal cell strains derived from a bronchogenic carcinoma. Proc. Natl. Acad. Sci. USA (in press)
3. Albert, D.M., Tso, M.O.M., and Rabson, A.S.: In vitro growth of pure cultures of retinal pigment epithelium. Arch. Ophthal. 88: 63-69, 1972
4. Albert, D.M., Tso, M.O.M., and Rabson, A.S.: Experimental malignant tumors from retinal pigment epithelium. Arch. Ophthal. 88: 70-74, 1972

5. Albert, D.M., and Rabson, A.S.: The role of viruses in the pathogenesis of ocular tumors. In Ferry, A.P. (Ed.): Ocular and Adnexal Tumors. (International Ophthalmology Clinics). Boston, Mass., Little, Brown and Company, 1972, vol. 12, no. 1, pp. 195-224
6. Paulson, D.F., Fraley, E.E., DeKernion, J.B., and Rabson, A.S.: Virus-induced genitourinary malignancy. Macromolecular identity. Invest. Urol. 10: 298-304, 1973

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 531

Title: In Vitro Studies of Human Malignant Tumors

Principal Investigator: Alan S. Rabson, M.D.

Section: Viral Oncology and Molecular Pathology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To isolate and characterize oncogenic viruses from human cancers.

Approach: Application of virological techniques successful in isolation of animal tumor viruses to human cancer cells.

Progress: Cell lines derived from human lung cancer, carcinoma of the uterine cervix and retinoblastoma are now well established. Viral isolation studies are in progress.

Significance for Cancer Research (NCP Objective 3 Approach 2)

Level of Effort: Professional Man-Years: 2/3

Serial No. NCI 532

1. Laboratory of Pathology
2. Viral Oncology and Molecular Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Oncogenic DNA Viruses

Previous Serial Number: Same

Principal Investigator: Alan S. Rabson, M.D.

Other Investigators: N. Munoz, E. Chu, R.L. Kirschstein, G.T. O'Connor, and
P. Periman

Cooperating Units: None

Man Years (July 1, 1972 - June 30, 1973)

Total: 3/4

Professional: 1/2

Other: 1/4

Project Description:

Objectives: To study some biological properties of DNA viruses with proven or possible oncogenic activity.

Methods Employed: Standard virologic and pathologic methods have been used.

Major Findings: Work has continued with the model system of Herpesvirus saimiri in marmoset cells. The system continues to show remarkable similarity to the EB virus in man. During the summer of 1972, Dr. George Klein of the Karolinska Institute was a visiting scientist in our laboratory and in collaboration with Drs. Gary Pearson and D. Ablashi of NCI we have studied antibodies to early and late antigens of H. saimiri in marmosets, owl monkeys and squirrel monkeys with and without induced lymphomas. The results are similar to those found in EB infection in man with antibody to the late antigens present in squirrel monkeys after natural infection and antibodies to both early and late antigens in marmosets and owl monkeys with tumors. In the squirrel monkeys infected in the laboratory, antibodies to both early and late antigens appear during infection but those to the early antigens regress while those to the late antigens persist.

In collaboration with Dr. Dolin of NIAID we have found that H. saimiri provides a partial helper function for the defective adeno-associated viruses of man.

As part of our program to study mechanisms of latency of herpesviruses and their possible oncogenic potential in man, we have studied human trigeminal ganglia removed at autopsy for presence of Herpesvirus hominis and have succeeded in demonstrating latent virus in 2 of 22 patients.

We have been continuing our studies of human cytomegaloviruses and their possible relationship to human cancer. Thus far we have no evidence for presence of cytomegalovirus or its antigens in any of the tumors we have examined.

Significance: The mechanisms of repression and possible integration of herpesviruses in mammalian cells and mechanisms of neoplastic transformation may be of extreme importance in understanding the role of herpesviruses in human cancer. Studies with a model system involving a primate oncogenic herpesvirus such as H. saimiri and studies of herpesviruses in human lymphoma cells may be of value in this regard.

Proposed Course: We intend to continue studies of the virus-cell relationship in the marmoset lymphoid cell line and to study factors involved in activation of the viral genome. We also intend to continue studies of the mechanisms of repression of herpesviruses in both lymphoid and non-lymphoid cell systems.

Publications:

1. Chu, E.W., and Rabson, A.S.: Chimerism in lymphoid cell culture line derived from lymph node of marmoset infected with Herpesvirus saimiri. J. Natl. Cancer Inst. 48: 771-775, 1972
2. Periman, P., Tyrrell, S.A., and Rabson, A.S.: Herpesvirus saimiri in marmoset-mouse hybrid cell lines. J. Natl. Cancer Inst. 49: 387-393, 1972
3. Dolin, R., and Rabson, A.S.: Herpesvirus saimiri: Enhancement of adenovirus-associated virus. J. Natl. Cancer Inst. 50: 205-208, 1973
4. Klein, G., Pearson, G., Rabson, A.S., Ablashi, D.V., Falk, L., Wolfe, L., Deinhardt, F., and Rabin, H.: Antibody reactions to Herpesvirus saimiri (HSV)-induced early and late antigens (EA and LA) in HSV-infected squirrel, marmoset and owl monkeys. Int. J. Cancer (in press)
5. Bastian, F.O., Rabson, A.S., Yee, C.L., and Tralka, T.S.: Herpesvirus hominis: Isolation from human trigeminal ganglion. Science 178: 306-307, 1972
6. Worthington, M., Rabson, A.S., and Baron, S.: Mechanism of recovery from systemic vaccinia virus infection. I. The effects of cyclophosphamide. J. Exp. Med. 136: 277-290, 1972

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI- 532

Title: Studies on Oncogenic DNA Viruses

Principal Investigator: Alan S. Rabson, M.D.

Section: Viral Oncology and Molecular Pathology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To characterize virus-cell relationships with known oncogenic animal herpesviruses such as Herpesvirus saimiri, and to attempt to identify human herpesviruses in human cancers.

Approach: The H. saimiri model system is being studied in vitro with cultures of transformed lymphoid cells derived from animals with H. saimiri induced lymphomas. The methods useful in demonstrating and activating H. saimiri virus and viral antigens are being applied to human cancer cells.

Progress: Lymphoblastoid cell line from a marmoset with an H. saimiri induced lymphoma has been established and the virus-host cell relationship has been studied. Herpesvirus hominis has been isolated from human trigeminal ganglia of 2 of 22 patients by cocultivation methods successful in recovery of H. saimiri from tumor cells and attempts to isolate herpesviruses from human cancer cells with similar methods are in progress.

Significance for Cancer Research (NCP Objective 3 Approach 2)

Level of Effort: Professional Man-Years: 1/2

Serial No. NCI 580

1. Laboratory of Pathology
2. Viral Oncology and Molecular Pathology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: The Virus-Host Relationship in Mixed Murine Leukemia Virus-Arbovirus Infection

Previous Serial Number: Same

Principal Investigator: Judith G. Levin, Ph.D.

Other Investigators: P.M. Grimley, J. Dalrymple, and W. Brandt

Cooperating Units: Walter Reed Army Institute of Research

Man Years (July 1, 1972 - June 30, 1973)

Total: 2 1/2

Professional: 2

Other: 1/2

Project Description:

Objectives: 1. To study the effect of exogenous infection with a non-oncogenic RNA virus on cells chronically infected with murine leukemia virus. 2. To identify host cell factors which may be required for viral RNA and protein synthesis. 3. To study host cell involvement in group A arbovirus infection.

Methods Employed: The methods employed include growth of cells in tissue culture; growth of arbovirus in various cell lines and determination of virus titer by plaque assay; preparation of cell-free extracts for assay of viral RNA polymerase activity; polyacrylamide gel electrophoresis of viral proteins and RNA species extracted from infected cells; electron microscopy; determinations of leukemia virus production by assay of reverse transcriptase activity in extracellular fluids; and immunological assays e.g. complement-fixation test.

Major Findings: 1. Four murine cell lines were examined in this study: two L cell lines; JLS-V9 cells, chronically infected with Rauscher leukemia virus; and a leukemia virus-free line of AKR cells. Production of C-type virus was determined in each of the lines by means of electron microscopy, immunological assay for the murine leukemia virus group-specific antigen, and measurement of reverse transcriptase activity in the extracellular fluids. When the cells were infected with Semliki Forest virus (SFV), a group A arbovirus, it was found that the SFV yields were very similar in the leukemia

virus producer and non-producer lines. This finding indicates that replication of the arbovirus is unaffected by chronic infection of murine cells with murine leukemia virus.

2. Ultrastructural characterization of the mixedly infected cells (in collaboration with Dr. P. Grimley) indicated that budding and release of C-type virus continue throughout the replicative cycle of the arbovirus.

3. (a) Arbovirus infection of murine cells was characterized and compared with the chick embryo fibroblast system (heretofore the standard tissue culture system used for growth of SFV and other group A arboviruses). The data indicated many similarities. Thus, identical viral RNA and protein species were found, and the kinetics of viral RNA synthesis, levels of viral RNA polymerase, and viral morphogenesis were quite similar.

(b) Serial passage of SFV in chick embryo fibroblasts had only a small effect on SFV yield. However, when murine cells were infected at high multiplicity with a late passage pool from chick cells, the virus yield was reduced by ten-to-twenty-fold and there was a marked alteration in the proportions of intracellular viral RNA species. These results are consistent with the possibility that defective interfering SFV particles were produced during infection with the late passage pool from chick cells. Since infection of chick cells with the same virus pool had a much less dramatic effect on viral RNA synthesis and viral yield, it appears that formation of defective arbovirus particles is more readily detected in a murine host. Further evidence for the accumulation of defective SFV in a mammalian host was obtained from studies on SFV infection of HeLa cells.

Significance: 1. The availability of a system in which a non-oncogenic RNA virus can superinfect cells chronically infected with an RNA tumor virus presents a unique opportunity to study questions pertaining to the status of the tumor virus in relation to the host. Current theories of oncogenesis postulate that in cells continuously producing leukemia virus, the viral genome is integrated into the DNA of the host. Since SFV suppresses host macromolecular synthesis, it is of interest to assess the influence of the arbovirus on expression of the leukemia virus genome.

2. The studies on defective arbovirus particles are important because production of defective virus represents one of the mechanisms by which a host can limit virus infection and can be a major factor in determining whether a given infection is permissive or restrictive. A clearer understanding of this phenomenon should find application in studies on chronic virus diseases. In addition, the work is relevant to the RNA tumor virus systems where it is known that defective particles are produced, to a greater or lesser extent (depending on the particular host cell), with continuous passage in culture.

Proposed Course: 1. Studies dealing with the effect of SFV on chronic leukemia virus infection e.g. the extent of leukemia virus production during SFV infection will be determined by using the appearance of reverse transcriptase activity in the culture fluids to estimate release of tumor virus particles.

2. Immunological studies utilizing rabbit anti-SFV serum to determine whether a mixed arbovirus-murine leukemia virus infection results in the release of leukemia virus particles which contain arbovirus antigen(s).

3. Further studies on defective SFV particles--in particular to attempt to separate defective and standard virus and to characterize the RNA species contained in the defective particles.

Publications:

1. Grimley, P.M., Levin, J.G., Berezesky, I.K., and Friedman, R.M.: Specific membranous structures associated with the replication of group A arboviruses. J. Virol. 10: 492-503, 1972
2. Friedman, R.M., Levin, J.G., Grimley, P.M., and Berezesky, I.K.: Membrane-associated replication complex in arbovirus infection. J. Virol. 10: 504-515, 1972
3. Grimley, P.M., Berezesky, I.K., and Levin, J.G.: Morphogenesis of C-type and A-type particles in cells infected by an arbovirus. J. Natl. Cancer Inst. 50: 275-279, 1973

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-580

Title: The Virus-Host Relationship in Mixed Murine Leukemia Virus-Arbovirus Infection

Principal Investigator: Judith G. Levin, Ph.D.

Section: Viral Oncology and Molecular Pathology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To study the virus-host relationship in chronic infection with murine leukemia virus and the effect of exogenous infection with a non-oncogenic RNA virus.

Approach: Standard tissue culture and virological techniques were employed to grow cells and to titer the arbovirus. Production of leukemia virus was determined by means of electron microscopy, immunological assay for the murine leukemia virus group-specific antigen, and measurement of reverse transcriptase activity in the extracellular fluids. Viral RNA and protein species were analyzed by polyacrylamide gel electrophoresis.

Progress: Infection of cells with a group A arbovirus resulted in similar virus yields in murine leukemia virus producer and non-producer lines. This indicated that replication of the arbovirus is unaffected by chronic infection of murine cells with murine leukemia virus. Ultrastructural characterization of the mixedly infected cells showed that budding and release of C-type virus continue throughout the replicative cycle of the arbovirus. The arbovirus infection in murine cells was characterized biochemically. Conditions were found which favor formation of defective interfering arbovirus particles in these cells.

Significance for Cancer Research (NCP Objective 3 Approach 2)

The availability of a system in which a non-oncogenic RNA virus can super-infect cells chronically infected with leukemia virus presents a unique opportunity to study questions pertaining to the status of the tumor virus in relation to the host and to elucidate factors which influence expression of the leukemia virus genome.

Level of Effort: Professional Man-Years: 2

Serial No. NCI 587

1. Laboratory of Pathology
2. Viral Oncology and Molecular Pathology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title: Morphogenesis of Murine Leukemia Virus

Previous Serial Number: None

Principal Investigator: Judith G. Levin, Ph.D.

Other Investigators: P.M. Grimley

Cooperating Units: None

Man Years (July 1, 1972 - June 30, 1973)

Total: 2

Professional: 1 1/2

Other: 1/2

Project Description:

Objectives: 1. To study the mechanism of assembly of murine leukemia virus. 2. To characterize the leukemia virus particles produced under various metabolic conditions.

Methods Employed: The methods employed include growth of cells in tissue culture; determination of leukemia virus production by assay of reverse transcriptase activity in extracellular fluids; immunological assays; purification of leukemia virus by sucrose density gradient centrifugation; analysis of viral RNA by polyacrylamide gel electrophoresis; and electron microscopy.

Major Findings: 1. Using murine leukemia virus producer and non-producer cell lines, it was found that the presence of reverse transcriptase activity in the extracellular fluids correlated with high CF titer for the murine leukemia virus group-specific antigen and electron microscopic detection of C-type virus. Thus, the enzyme assay could be used as an index of leukemia virus production.

2. Under conditions where actinomycin D inhibited cellular and viral RNA synthesis by at least 90%, high levels of reverse transcriptase activity were detected in the extracellular fluids and C-type virus particles were frequently observed budding from cell surfaces. Since there has been no ultrastructural evidence for the existence of pre-formed viral core particles, these findings imply that murine leukemia virus-infected cells contain a

substantial pool of macromolecular components which can be utilized for terminal assembly of the virus.

3. Particles released from actinomycin D-treated cells sedimented in a sucrose gradient with a buoyant density characteristic of the RNA tumor viruses (1.15 - 1.16 g/cm³).

Significance: 1. The detailed mechanisms involved in tumor virus assembly are not fully known. This information is needed for a clearer understanding of tumor virus replication and may provide new approaches for developing drugs which inhibit virus production.

2. Model systems such as the one utilized in this study are important for attempts to delineate a viral etiology in human cancer. For example, such systems permit controlled manipulation of the experimental conditions to an extent which would not be possible in man or even in intact animals.

Proposed Course: 1. Characterization of the leukemia virus particles produced in the presence of actinomycin D including determination of RNA and protein species contained in the particles; biological activity (e.g. infectivity in XC assay); and immunological properties.

2. Use of other metabolic inhibitors to study terminal steps in virus assembly.

INTRAMURAL RESEARCH SUMMARY

Serial Number: NCI-587

Title: Morphogenesis of Murine Leukemia Virus

Principal Investigator: Judith G. Levin, Ph.D.

Section: Viral Oncology and Molecular Pathology

Lab/Branch: Laboratory of Pathology, DCBD, NCI

Goal: To study the development of murine leukemia virus in tissue culture systems.

Approach: Cells chronically infected with murine leukemia virus were used. Production of leukemia virus was determined by means of electron microscopy, immunological assay for the group-specific antigen, and measurement of reverse transcriptase activity in the extracellular fluids. Virus particles were purified from fluids by equilibrium sedimentation in sucrose density gradients. Viral RNA was analyzed by polyacrylamide gel electrophoresis.

Progress: The effect of actinomycin D on production of murine leukemia virus was examined. Under conditions where actinomycin D inhibited cellular and viral RNA synthesis by at least 90%, high levels of reverse transcriptase activity were detected in the extracellular fluids and C-type virus particles were frequently observed budding from cell surfaces. The particles released from actinomycin D-treated cells sedimented in a sucrose gradient with a buoyant density characteristic of the RNA tumor viruses ($1.15 - 1.16 \text{ g/cm}^3$).

Significance for Cancer Research (NCP Objective 3 Approach 2)

An understanding of the basic mechanisms involved in tumor virus production is important for developing methods to prevent neoplastic transformation of normal cells and for interrupting the process once it has begun.

Level of Effort: Professional Man-Years: 1 1/2

TUMOR IMMUNOLOGY CONTRACT PROGRAM
July 1, 1972 through June 30, 1973

An Immunology Program Plan has been designed which has as its goals 1) the use of immunologic techniques to detect agents etiologic for cancer, 2) the prevention of cancer by immunologic manipulations, 3) the use of immunologic techniques to improve the diagnosis of cancer, and 4) to manipulate the immune system of patients with established cancer so that increased survival is achieved.

The Immunology Contract Program has been designed to achieve these goals through the support of investigations relevant to the Plan. Thus far, implementation has been directed toward 1) studies of fundamental problems (designated immunobiology), the solutions to which are required for achieving the four goals, 2) immunodiagnosics, and 3) immunotherapy. All projects have been assigned to one of these three categories although many projects include research in more than one category.

A. Immunobiology

1. Studies of the nature of cell surfaces of tumor cells and cells of the immune system. Projects in this area include studies of 1) tumor associated antigens of human (69-2176, CB-33876*) and animal (70-2320) tumors, 2) genetically determined antigens on human lymphoid cells (69-2177), 3) the nature of tumor cell surfaces (72-3884, CB-33879*), and 4) the utilization of cell surface characteristics to permit separation of cells into different populations at the preparative level (72-3888).

2. Experimental aspects of interactions between tumors and the immune system of tumor bearing hosts, and identification of immune cells involved in reactions with tumor cells. Projects in this area include studies to 1) develop reagents for the identification of human T and B cells (72-3882), 2) determine the roles of different cell populations in immune reactions against tumors (72-3889, CB-33859*, CB-33866*, CB-33868*, CB-33870*), 3) explore the role of thymic hormones in immune reactions against tumors (72-3890-4), 4) determine the role of the immunoglobulin coating tumor cells (CB-33867*), and 5) assess multiple aspects of the interactions between tumor cells and the immune system (70-2207 and 72-3878).

3. Development and supply of tumors and tumor cells, and studies of cell growth and differentiation. Projects in this area include 1) the establishment of libraries of murine tumor cell lines for support of tumor immunology research (G-72-3886, G-69-2142), 2) studies of serum factors supporting and inhibiting in vitro cell growth (72-3883), and 3) studies of factors controlling differentiation of tumor cells (72-3890-3, CB-33854*).

B. Immunodiagnosis

* Approved but not funded.

1. The use of carcinofoetal antigens in the diagnosis of human cancer. Projects in this area include 1) attempts to develop more sensitive assays (72-3890-2), 2) attempts to structurally characterize carcinofoetal proteins (72-3877, CB-33869), and 3) clinical studies of the usefulness of various assays in the diagnosis or monitoring of patients with malignancy (73-3875, CB-33858*, G-71-2337, 72-3881, 73-3852).

2. The development of new tests for diagnosis. Projects include 1) test development and application (72-3227, 73-24*, 73-25*, 73-26*) and 2) acquisition of resources needed for evaluating new procedures (72-3879, 73-19*, 73-22*, 73-23*, and 73-27*).

C. Immunotherapy

1. Studies of immunotherapy in animal models. Projects include studies of 1) the role of different cell populations in immune reactions to tumors (72-3890-1), 2) the usefulness (CB-33853) and management (70-53 and 70-2103) of graft versus host disease in cancer therapy, 3) optimal methods for using neuraminidase treated cells in the therapy of cancer (73-21A* and B*) and possible mechanisms involved (CB-33860*), and 4) immunostimulants in the treatment of animal models with minimal residual disease (73-20*).

2. Application to immunotherapy in man. Projects include studies of 1) the usefulness of BCG in disseminated human malignancies (73-15*), in lung cancer (73-16*), and in melanoma with minimal residual disease (73-17*), 2) the possible usefulness of "deblocking" serum in patients with melanoma (72-3887), 3) the use of neuraminidase treated autologous cells in a number of malignancies (72-3885), and 4) the growth of allogeneic cells for preparation of treatment vaccines in leukemia (71-2078).

* Approved but not funded.

CONTRACT RESEARCH SUMMARY

Title: NCI Human Histocompatibility Typing Center

Principal Investigator: Dr. R. J. Trapani
Name/Address Microbiological Associates, Inc.
Performing 4733 Bethesda Avenue
Organization: Bethesda, Maryland 20014

Contract Number: NIH-NCI-G-69-2177

Starting Date: June 16, 1969

Expiration Date: June 15, 1973

Goal:

SEE ATTACHED SUMMARIES

Approach:

Progress:

Significance for Cancer Research (NCP Objective Approach)

Project Officer: G. Nicholas Rogentine, Jr., M.D., Dean L. Mann, M.D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Tumor Immunology Ad Hoc Committee, 3/30/73
Relevance Review Group: Waived
FY73 Funds: \$217,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: NCI Human Histocompatibility Typing Center

Principal Investigator: Dr. R. J. Trapani
Name/Address: Microbiological Associates, Inc.
Performing: 4733 Bethesda Avenue
Organization: Bethesda, Maryland 20014

Contract Number: NIH-NCI-G-69-2177

Starting Date: June 16, 1969

Expiration Date: June 15, 1973

Goal: To assay solubilized HL-A alloantigens provided by the Project Officer. In addition, the solubilized and isolated HL-A alloantigens are being used to immunize animals for development of heterologous sera that has antibody directed against these components.

Approach: The assay for solubilized HL-A alloantigen utilizes a cytotoxic inhibition assay. Target cells bearing the HL-A alloantigenic determinants to be analyzed are labeled with ^{51}Cr . The solubilized materials are incubated with the solubilized antigenic components and the inhibition of reaction of these antisera to the target cell demonstrates the presence of this antigenic material.

Progress: The contractor has provided assays for the presence of HL-A alloantigenic materials provided by the Project Officer. In addition, heterologous antisera which precipitate these antigens have been raised by the contractor. The contractor has also begun looking at sera from patients with acute leukemia for the presence of antibody detecting an antigen associated with acute leukemia and/or antibody directed against HL-A alloantigens. Attempts are being made to differentiate and identify the development of antibody to acute leukemia associated antigens in patients with this disease.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Development of methodologies in the identification of isolated cell surface antigens is a necessary prerequisite to the identification of tumor specific associated antigens.

Project Officer: Dean L. Mann, M.D.

Program: Immunobiology

Site Visit Date: 4/18/69

Technical Review Group: Tumor Immunology Ad Hoc Committee, 3/30/73

Relevance Review Group: Waived

FY73 Funds:

CONTRACT RESEARCH SUMMARY

Title: NCI Human Histocompatibility Typing Center

Principal Investigator: Dr. R. J. Trapani
Name/Address: Microbiological Associates, Inc.
Performing: 4733 Bethesda Avenue
Organization: Bethesda, Maryland 20014

Contract Number: NIH-NCI-G-69-2177

Starting Date: June 16, 1969

Expiration Date: June 15, 1973

Goal: The goal of this contract is to study HL-A and other cell surface antigens on normal and neoplastic cells and their subcellular fractions.

Approach: Various serological tests, including cytotoxicity, C' fixation and agglutination are used to assay HL-A and other antigens on human white blood cells and tissue culture cells, both malignant and normal. Quantitative absorption techniques are also employed to quantify antigens on cells and subcellular particles. Lymphocytes of patients with various malignant diseases and immunological diseases are assayed to determine associations between these antigens and disease susceptibility. Subcellular particles of human lymphoid cells are studied for presence of HL-A antigens. These subcellular particles are used to immunize non-human animal species in attempts to develop reagents for typing human cells. The resultant antisera are tested for HL-A and other cell surface antibodies.

Progress: Several significant associations between HL-A antigens and disease categories have been found. The diseases include acute lymphocytic leukemia, Hodgkin's disease, gluten sensitive enteropathy and dermatitis herpetiformis. Heterologous anti HL-A antisera have been produced in rabbits.

Significance for Cancer Research (NCP Objective 3 Approach 5)
The finding of significant associations between cancer and HL-A antigens has relevance for understanding the nature of oncogenesis and for identifying those individuals particularly susceptible to cancer.

Project Officer: G. Nicholas Roentine, Jr.
Program: Immunobiology
Technical Review Group: Tumor Immunology Ad Hoc Committee, 3/30/73
Relevance Review Group: Waived
FY73 Funds:

CONTRACT RESEARCH SUMMARY

Title: In Vitro Tests for Tumor Specific Antigens, Antibodies and Immune Cells with Animal and Human Serum Cells

Principal Investigator: Dr. Edward Matthews
Name/Address: Litton Bionetics, Inc.
Performing: 7315 Wisconsin Avenue
Organization: Bethesda, Maryland 20014

Contract Number: NIH-NCI-G-70-2207

Starting Date: May 28, 1970

Expiration Date: May 27, 1974

Goal: To prepare cultured melanoma cells for immunotherapy vaccine, to study development and function of cell mediated immune cytotoxicity in humans, and to assay viral infected human cell lines for leukemia associated antigens.

Approach: 1) Grow human melanoma cells free of PPL. Treat with neuraminidase and freeze under sterile conditions. Test for sterility and pyrogenicity. Provide for human immunotherapy. Also, adapt for culture explants of NCI melanoma patients. 2) Study direct cellular cytotoxicity of human lymphocytes. Evaluate lymphocyte dependent antibody with ^{51}Cr release assays. Immunize normal human peripheral blood lymphocytes in vitro. Study antitumor immunity in mice with progressively growing lethal tumors. 3) Murine leukemia viruses are used to infect human nonlymphoid cell lines. Cells are assessed for acute leukemia associated antigen. Cell membranes are solubilized and analyzed by gel electrophoresis.

Progress: 1) Two cell lines are growing, one PPL free and one being converted to growth in human serum. 2) Human peripheral blood lymphocytes have been immunized in vitro. Human lymphocyte dependent antibody and cytotoxic cells have been further characterized. 3) Some cell lines infected with Rauscher leukemia have demonstrated the presence of antigen cross reactive with leukemia associated antigen. Antigen is associated with membranes and new cell membrane proteins have been identified.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Melanoma cell line material will be used for a clinical immunotherapy trial. In vitro immunization may boost an inadequate or blocked immune system. Leukemia associated antigen may be useful in finding etiologic agent for human leukemia.

Project Officer: Drs. William D. Terry, Dean L. Mann, John R. Wunderlich
Program: Immunobiology Site Visit Date: April 3, 1970
Technical Review Group: Tumor Immunology Ad Hoc Group of March 30, 1973
Relevance Review Group: Waived
FY73 Funds: \$419,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Study of the Immune Responses of Mice and Rats to Tumor Associated Antigens

Principal Investigator: Dr. David Lavrin
Name/Address: Litton Bionetics, Inc.
Performing: 5510 Nicholson Lane
Organization: Kensington, Maryland 20795

Contract Number: NIH-NCI-72-3878

Starting Date: 5/28/72

Expiration Date:

Goal: Study of immune responses of mice and rats to tumor associated antigens. Studies on in vivo resistance to tumors and correlation with in vitro cellular and humoral immune responses. Approach: The kinetics of the cellular and humoral immune responses to tumor associated antigens of the following tumors will be studied: tumors induced by polyoma virus, SV40, Gross and other murine leukemia viruses, murine sarcoma virus, and mammary tumor virus; mouse plasma cell tumors; methylcholanthrene-induced tumors. The relationships between the detected antigens and viral gene expression and expression of fetal antigens will be extensively studied.

The specificity of each of the detected antigens in both the humoral and cell-mediated immune studies are being intensively studied, by inhibition assays. The nature of the cells involved and the mechanism of cytotoxicity in the cell-mediated cytotoxicity assays are also being studied.

Progress: A major effort has been put into a continuing study of murine sarcoma virus (MSV)-induced cellular and humoral immunity in mice. The kinetics of the immune response and the specificity of the detected antigens have been defined. The attacker cells in ^{51}Cr cell-mediated cytotoxicity were shown to be theta-antigen positive lymphocytes. The effects of various immunosuppressive agents were also studied. The relative immunogenicity of fetal tissue and various DNA virus tumors to subsequent challenge with tumor cells was studied. Studies with mouse mammary tumors have been initiated.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Understanding of relationship of in vitro assays to host resistance to tumors is an essential element in properly using these assays to monitor response to immunotherapy.

Project Officer: Ronald B. Herberman, M.D.

Program: Immunology:Immunobiology Site Visit Date: 3/70

Technical Review Group: Ad Hoc Tumor Immunology Review Group

Relevance Review Group: Waived

FY73 Funds: \$274,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Prototype Production of Dual Parameter Cell Sorter

Principal Investigator: Leonard A. Herzenberg, Ph. D.
Name/Address: Leland Stanford Junior University
Performing: Stanford, California 94305
Organization:

Contract Number: NIH-NCI-G-72-3888

Starting Date: June 15, 1972

Expiration Date: April 30, 1973

Goal: To permit the production of two copies of the dual parameter cell sorter presently operative at Stanford University.

Approach: Two prototypes of this machine will be produced by the B-D Corporation on a subcontract to Stanford University. One of these machines will be left at Stanford for use by Dr. Leonard Herzenberg for immunology studies. The other machine will be delivered to the National Cancer Institute for use to be determined by the Project Officer.

Progress: Cell separators are being constructed as scheduled. The contract has been extended until October 1973 to permit installation of a dual window extractor which doubles machine efficiency. This contract will not be renewed.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Ability to separate lymphocytes from cancer patients will permit selective stimulation of immunocompetent cells against tumor, study of patients' responsiveness to tumor, and transfusion of selected cell populations.

Project Officer: William D. Terry, M. D. and John R. Wunderlich, M. D.

Program: Immunobiology

Site Visit Date: April 7, 1972

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: None

CONTRACT RESEARCH SUMMARY

Title: Development of Antisera Specific for Human T and B Lymphocytes

Principal Investigator: Max D. Cooper, M. D.
Name/Address: University of Alabama in Birmingham
Performing: The Medical Center
Organization: Birmingham, Alabama 35233

Contract Number: NIH-NCI-G-72-3882

Starting Date: June 20, 1972

Expiration Date: June 1974

Goal: To develop two reagent antisera, one specifically capable of recognizing B lymphocytes and the other capable of reacting specifically with T lymphocytes.

Approach: The contractor will develop these antisera by immunizing rabbits and goats with presumed T lymphocytes obtained from patients X-linked agammaglobulinemia as well as fetal thymus cells. Animals will be immunized with B lymphocytes obtained from patients selectively deficient in T lymphocytes, such as patients with chronic lymphocytic leukemia. Antisera will be appropriately absorbed and specificity tested by cytotoxic and binding activities for appropriate lymphoid cells.

Progress: Several rabbit antisera to human T lymphocytes from fetal thymus or peripheral leukocytes of an agammaglobulinemic donor have been prepared and extensively absorbed. Similar antisera have been raised against lymphocytes rich in B cells, but these are technical difficulties in the absorption of this material. Studies in patients with immunodeficiency and lymphoproliferative diseases are underway.

Significance for Cancer Research (NCP Objective 2 Approach 1)
Manipulation of T and B lymphocytes is a crucial aspect of developing reliable methods of altering immunologic capacity of individuals.

Project Officer: William D. Terry, M. D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Tumor Immunology Scientific Review Group
Relevance Review Group: Immunology Advisory Group
FY73 Funds: \$39,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Studies on Differentiation-inducing Factors for Leukemic Cells on Distinctive Chemical Structures on the Surface Membrane of Malignant Cells and on the Applicability of the Resulting Formation to Chemotherapy

Principal Investigator: Dr. Leo Sachs

Name/Address

Performing Weizmann Institute of Science

Organization: Rehovot, Israel

Contract Number: NIH-NCI-G-72-3890

Starting Date: June 26, 1972

Expiration Date: June 25, 1973

Goal: To explore the use of soluble factors for the induction of a normal pattern of cellular differentiation in leukemic cells.

Approach: The contractor will obtain sufficient quantities of soluble factors capable of altering cellular differentiation in vitro and will study the capacity of these factors to alter the morphologic pattern and functional characteristics of leukemic cells in vitro and in vivo.

Progress: Inadequate amounts of soluble factors were obtained to perform most of the studies intended. Sufficient amounts were obtained to reproduce the previous studies, demonstrating in vitro efficacy in the induction of differentiation of normal blast cells to mature granulocytes or macrophages and to indicate that adenine can substitute for the co-factor required for expression of activity.

Significance for Cancer Research (NCP Objective 6 Approach 4) It is possible that some forms of leukemia are due to a failure in cellular differentiation. Treatment of such leukemias might be possible if differentiation-inducing factors would act in vivo.

Project Officer: Drs. William Terry, Harry Gelboin, Donald Buell, Pierre

Program: Immunobiology

Site Visit Date: Nov. 1972 Henkart

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: none

CONTRACT RESEARCH SUMMARY

Title: Analysis of Serum Requirements for In Vitro Immunological Studies

Principal Investigator: Robert I. Mishell, M. D.
Name/Address: University of California, Berkeley
Performing: Berkeley, California 94720
Organization:

Contract Number: NIH-NCI-G-72-3883

Starting Date: June 28, 1972

Expiration Date: June 1974

Goal: To define serum requirements for immunological studies employing tissue culture techniques.

Approach: Using the mouse spleen in vitro immune response as an assay, sera obtained from several species will be examined for their capacity to substitute for fetal bovine sera. The latter are in very limited supply. In addition, attempts will be made to explore the use of bacterial endotoxin and other agents for their capacity to restore batches of sera that are not useful in the in vitro assays. Furthermore, preliminary fractionation and characterization of both toxic and growth promoting substances will be carried out.

Progress: Sixty-seven lots of fetal bovine, calf, adult bovine, mouse, lamb, and swine sera have been tested for effects on both primary and secondary response in vitro, crude endotoxin and Concanavalin A have been tested and found to restore 16 lots of deficient fetal bovine sera. Complementation tests have been performed on 13 lots of sera, and an initial fractionation has been performed to isolate the supportive activity of positive sera.

Significance for Cancer Research (NCP Objective 4 Approach 1)
Most in vitro studies of cellular immune processes depend on added serum. It is essential that sources of such sera be standardized in order to properly evaluate cancer patients' responses to therapy.
Project Officer: William D. Terry, M. D.
Program: Immunobiology
Technical Review Group: Tumor Immunology Scientific Review Group
Relevance Review Group: Immunology Advisory Group
FY73 Funds: \$75,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Characterization of Phytocytoagglutinins

Principal Investigator: Donald D. Poretz, Ph.D.
Name/Address: University of Kansas Medical Center
Performing: Kansas City, Kansas 66103
Organization:

Contract Number: NIH-NCI-G-72-3884

Starting Date: June 28, 1972

Expiration Date: September, 1973

Goal: To obtain in a reasonable state of purity a number of lectins for use in studies of the relationship between the cell membranes of normal and malignant cells.

Approach:

Utilizing specific absorption procedures and/or classic chemical separation techniques, the investigator will attempt to purify plant lectins in sufficient quantities to make them available to other investigators for studies of the surfaces of malignant cells. The estimation of functional purity will be evaluated in terms of cytoagglutinin properties and specificities toward carbohydrates.

Progress: The hemagglutinin from Wisteria floribunda has been purified and several hundred mg of this lectin have been prepared. Purity was established by gel electrophoresis and immunoelectrophoresis. Inhibition of agglutinin was achieved with α and β galactosides and N-acetyl galactosamine. Techniques for the purification of Labrunum alpinum are being developed. This lectin, which preferentially agglutinates type O human red blood cells, is currently 90-95% pure.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Cell surfaces are recognized to hold the secret to understanding malignant growth. Lectins are an important tool for study of the problem.

Project Officer: Pierre A. Henkart, Ph.D.

Program: Immunobiology

Site Visit Date: May 3, 1972

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: No funds from FY73

CONTRACT RESEARCH SUMMARY

Title: Production of Human Lymphoid Cells in Culture

Principal Investigator: Dr. J. J. Oleson
Name/Address: Pfizer, Inc.
Performing: 199 Maywood Avenue
Organization: Maywood, New Jersey 07607

Contract Number: NIH-NCI-G-71-2078 (Formerly 70-2080)

Starting Date: January 1, 1971 Expiration Date: Dec. 1973

Goal: To grow and produce large quantities of human lymphoid cells in long term tissue culture and to grow the Raji cell line in human serum for immunization of acute leukemia patients.

Approach: Several human lymphoid tissue culture cell lines which bear different HL-A alloantigens are produced in large quantities and are isolated, concentrated, and shipped to the Project Officer for subsequent use in the isolation and characterization of the HL-A alloantigens. One of the cell lines, Raji, is being grown in quantities sufficient to initiate an immunization program in acute leukemia in humans. This cell line, unlike the other cell lines, is grown in normal human serum to eliminate the possibility of immunization with the fetal bovine serum foreign protein.

Progress: Several cell lines have been produced under this contract during the past year. The Raji cell line has been successfully grown in the human serum and has been and is currently being used to immunize patients with acute leukemia. In addition, this cell line is being used to isolate an antigen associated with acute leukemia. The products produced by this contract are being used to isolate and characterize the human HL-A alloantigens. In brief, membranes from these products are isolated, the HL-A alloantigenic components solubilized by detergent and papain methods, and purified by immunochemical as well as physical techniques.

Significance for Cancer Research (NCP Objective 3 Approach 5)

Provides prototype technique for the isolation of tumor specific or tumor associated antigens. Raji cell line has been used for immunotherapy in acute leukemia.

Project Officer: Dean L. Mann, M. D., William D. Terry, M. D.

Program: Immunobiology Site Visit Date: June 19, 1972

Technical Review Group: Tumor Immunology Advisory Group

Relevance Review Group: Waived per Dr. Berlin's memo of Feb. 15, 1973 to

FY73 Funds: estimate \$490,000

Dr. Terry

CONTRACT RESEARCH SUMMARY

Title: Facility for Supplying Immune Related Cell Lines

Principal Investigator: Dr. Melvin Cohn

Name/Address

Performing

The Salk Institute

Organization:

San Diego, California 92112

Contract Number: NIH-NCI-G-72-3886

Starting Date: June 26, 1972

Expiration Date: June 25, 1974

Goal: To establish a library of lymphoma and myeloma tumor cell lines and make these available to investigators.

Approach: The contractor will catalog the available cell lines in his laboratory and characterize them as regards their lymphoma and myeloma characteristics. The results will be put into a catalog and either frozen cell lines or mice carrying the appropriate tumors will be made available to investigators on demand.

Progress: Twenty-five culture lines and 36 tumor lines have been rechecked and made available as initial items for distribution. A catalog has been prepared and will be distributed. Requests for cell lines and tumors have been processed.

Significance for Cancer Research (NCP Objective 6 Approach 1) Experimental cancer immunology studies require cell lines and tumors of known characteristics. This contract provides a national resource which supports cancer research throughout the country.

Project Officer: William D. Terry, M.D.

Program: Immunobiology

Site Visit Date: April 19, 1972

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$100,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Supramolecular Organization of Normal and Tumor Cell Surfaces and Their Relationship to Escape from Immune Surveillance and Growth Control

Principal Investigator: Garth L. Nicholson, Ph. D.
Name/Address: The Salk Institute for Biological Studies
Performing: P. O. Box 1809
Organization: San Diego, California 92112

Contract Number: N01-CB-33879

Starting Date: Estimated June 1973 Expiration Date: June 1974

Goal: To examine quantity and spatial organization of cell surface antigens and saccharides in normal and malignant cells.

Approach: Normal and transformed cells in tissue culture will be reacted with radiolabeled antibodies and lectins and then studied with electron microscopic and cell fractionation procedures.

Progress: New contract

Significance for Cancer Research (NCP Objective 3 Approach 5)
Understanding cell surface changes underlies many approaches to cancer prevention.

Project Officer: William D. Terry, M. D.
Program: Immunobiology Site Visit Date:
Technical Review Group: Tumor Immunology Scientific Review Group
Relevance Review Group: Tumor Immunology Advisory Group
FY73 Funds: \$100,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Roles of Purified Cells and Antibodies in Cell-mediated Lysis

Principal Investigator: Dr. Hans Wigzell

Name/Address

Performing Karolinska Institute

Organization: S-104 01, Stockholm 60, Sweden

Contract Number: N01-CB-33859

Starting Date: In negotiation

Expiration Date:

Goal: To evaluate several different proposed methodologies for the purpose of achieving high concentrations of T cells and B cells independently of each other.

Approach: The following methodologies will be thoroughly evaluated: 1) the use of antigen coated bead columns to cause adherence of B cells with the subsequent use of dextranase to dissolve the beads, 2) selective cytolysis of T cells with specific anti-T cell antibody, and 3) the evaluation of monolayers of appropriate target cells to achieve adherence of specifically sensitized lymphocytes.

For monitoring, at least three target systems will be used, chick red blood cells, tumor cells (human or mouse), and histoincompatible fibroblasts or tumor cells.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 3 & 4)
6 4

The ability to work with purified populations of immunocompetent cells is basic to understanding and manipulating immune control of cancer.

Project Officer: William D. Terry, M.D.

Program: Immunobiology

Site Visit Date:

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$23,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: The Role of T-lymphocytes in Tumor-Associated Immunity

Principal Investigator: Dr. Birger Andersson
Name/Address Karolinska Institute
Performing S-104 01, Stockholm 60, Sweden
Organization:

Contract Number: N01-CB-33866
Starting Date: In negotiation Expiration Date:
Goal: To evaluate the role of B cell immunity in in vivo tumor rejection using specially prepared animals.

Approach: Tumor rejection capacity will be evaluated by inoculation of titrated tumor doses and measurement of antibody production. At least three well-defined tumors will be used in these assays.

Evaluation of T cell effects will be made utilizing at least two different methods of T cell reconstitution.

In utilizing bone marrow for B cell reconstitution, effort will be made to evaluate or eliminate the presence of contaminating T cells.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 2 & 3)
6 1 & 4

Understanding the role of types and relative numbers of T and B cells in immune reactions is basic to manipulation of immune defense against cancer.

Project Officer: William D. Terry, M.D.

Program: Immunobiology

Site Visit Date:

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$20,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Cell Synergy in Cell Bound Immune Reactions

Principal Investigator: Dr. Henric Blomgren

Name/Address

Performing Karolinska Institute

Organization: S-104 01, Stockholm 60, Sweden

Contract Number: N01-CB-33868

Starting Date: In negotiation

Expiration Date:

Goal: 1) To evaluate synergism between thymus cells and lymph node cells in graft versus host and tumor rejection reactions, and 2) to evaluate T cell tolerance after passage through allogeneic hosts.

Approach: Project 1 will evaluate the interrelations between thymocytes and lymph node cells in several situations as follows: a) synergistically in graft versus host responses, b) antagonistically in graft versus host responses, c) synergistic effects on the rejection of allogeneic tumors, and d) synergy in reactions between thymocytes and lymph node cells in the mixed lymphocyte reaction (MLR). In particular, the kinetics of regenerating thymus cells, their circulating characteristics, and their PHA responsiveness will be evaluated.

Project 2 will be a study of the development of immunologic tolerance in T cell populations passaged through allogeneic hosts. A second part of this project will be evaluation of the specific tolerance that can be seen in the donor bone marrow that is used to reconstitute lethally irradiated animals shortly after irradiation. In particular, the duration of tolerance will be evaluated and the question of whether bone marrow from such a chimera can be used to protect lethally irradiated mice syngeneic with the marrow will be examined.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 3 & 4)
6 1 & 4

Understanding the role of types and relative numbers of T and B cells in immune reactions, including tolerance, is basic to manipulation of immune defense against cancer.

Project Officer: William D. Terry, M.D.

Program: Immunobiology

Site Visit Date:

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$10,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Effector Mechanisms in Tumor-associated Immune Reactions

Principal Investigator: Dr. Eva Klein

Name/Address

Performing Karolinska Institute

Organization: S-104 01, Stockholm 60, Sweden

Contract Number: N01-CB-33870

Starting Date: In negotiation

Expiration Date:

Goal: To study the mechanisms of B cell action in tumors in six different model systems: 1) the role of antibody effect in cytotoxicity, 2) Burkitt lymphoma and mouse lymphoma in nude mice, 3) human B cells in relation to Burkitt cell in culture, 4) human glioma patients' lymphocytes in relation to glioma cell lines, 5) Marek's disease, and 6) evaluation of human chronic lymphatic leukemia cells for capacity to act as cytotoxic cells.

Approach: 1) Evaluate humoral antibody effect on the cytotoxicity for tumor of several classes of lymphocytes in which the lymphocytes will be separated by methods worked out in conjunction with Dr. Wigzell. This work will be performed in a mouse system and due regard will be given for H-2 incompatibility of host and tumor. 2) Evaluate B cell capacity to reject tumors by utilizing nude (thymusless) mice. The tumors to be studied shall include human B cells to attack Burkitt lymphoma cells as a function of the lymphoma cells' coating of IgG. 4) Study lymphocytes derived from patients with glioma for the kinetics of the lymphocyte blastogenic response to glioma cell lines. 5) Evaluate tumor specific antigens, surface immunoglobulin, and vaccination phenomena in Marek's disease. 6) Study human chronic lymphatic leukemia cells (presumably B cells) for their capacity to act as killer cells against antibody coated target cells.

Progress: New contract.

Significance for Cancer Research (NCP Objective 3 Approach 4)

6	1
6	4

Understanding immunocompetent cell behavior in malignant disease is basic to evaluating and manipulating immune defense against cancer.

Project Officer: William D. Terry, M.D.

Program: Immunobiology

Site Visit Date:

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$30,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Immunological and Virological Studies with Human Sarcomas

Principal Investigator: Dr. Donald L. Morton
Name/Address: University of California, Los Angeles
Performing: Center for the Health Sciences, Los Angeles, Calif.
Organization:

Contract Number: N01 - CB - 33876

Starting Date: In negotiation

Expiration Date:

Goal:

To examine tumor specific antigens of human sarcoma lines for evidence of viral agents, to define such viral agents, and to compare the antigens of human sarcomas and any possible viral agents with the known antigens and agents of animal tumors having RNA viruses.

Approach:

1) Collect tumor specimens and serum from sarcoma patients, 2) establish primary tissue culture of tumor and normal tissues of each patient, 3) utilize complement fixation with human serum, immunodiffusion, and microcytotoxicity tests for the comparison of antigens between tumor lines, 4) analyze cultures of tumor for the presence of filterable agents, 5) characterize such filterable agents for physical and chemical properties, host range, growth curve, and morphology, 6) attempt to recover a sarcoma virus utilizing co-cultivation experiments with human and animal embryo cells and the addition of helper viruses; additionally, irradiation and carcinogens will be used to attempt to activate possible latent viruses, 7) infect human cells with animal tumor viruses to compare any new antigens with those in human sarcoma cells.

Progress:

Newly approved contract

Significance for Cancer Research (NCP Objective 3 Approach 4)

The unique systems being utilized here provide new approaches to possible detection of causative agents in human cancer.

Project Officer: William D. Terry, M.D.

Program: Immunobiology

Site Visit Date: August 14, 1972

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Tumor Immunology Advisory Group

FY73 Funds: \$146,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Nature of Interactions Between Tumor Cells and Immunoglobulins

Principal Investigator: Dr. Isaac P. Witz
Name/Address: Tel Aviv University
Performing: Tel Aviv, Israel
Organization:

Contract Number: N01-CB-33867

Starting Date: In negotiation

Expiration Date:

Goal: This contractor will examine the role of natural and tumor specific immunoglobulin coating of tumor cells in relation to antibody mediated and lymphocyte mediated cytotoxicity.

Approach: The contractor will (1) prepare purified Ig from acid eluates of coated tumor cells; (2) examine ascites, T lymphoma, mammary carcinoma, and carcinogen-induced sarcoma cells for complement-dependent cytotoxicity, lymphocyte-mediated cytotoxicity and antibody-mediated cytotoxicity, using uncoated tumor cells mixed with the acid eluates, in vivo coated tumor cells, and uncoated tumor cells as model systems; (3) utilize mice who have been immunized with noncross-reactive antigens and then challenged with ascites tumor cells and examine whether these cells show the capacity to bind the radiolabeled immunizing antigens and its effect on cytotoxicity assays; and (4) study the surface binding capacity of cultured tumor cells for various immunoglobulin fractions obtained from normal mice or from plasmacytoma bearing mice.

Progress:

New contract

Significance for Cancer Research (NCP Objective 3 Approach 4)

The capacity of the immune system to recognize and eliminate transformed cells may depend on and be modified by immunoglobulins.

Project Officer: William D. Terry, M.D.

Program: Immunobiology

Site Visit Date:

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Tumor Immunology Advisory Group

FY73 Funds: \$35,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Immunodiagnostic Testing, Antibody Measurement and Fractionation of Serum and Urine

Principal Investigator: Dr. Roy Woods

Name/Address

Performing Meloy Laboratories, Inc.

Organization: Springfield, Virginia 22151

Contract Number: N01-CB-33875 (formerly PH43-NCI-G-66-3)

Goal: To use quantitative and qualitative immunochemical techniques for improved diagnosis of cancer and to evaluate humoral immune responsiveness of patients with cancer.

Approach: Qualitative and quantitative immunochemical techniques are used for the diagnosis of multiple myeloma, Waldenström's macroglobulinemia, agammaglobulinemia, and other diseases with immunoglobulin disorders. In addition, these same techniques are used to evaluate carcinoembryonic antigen and α -feto-protein in the serum of patients with a variety of malignancies. Studies are carried out to classify the abnormal proteins of the abnormality diseases and to study the α -feto-protein and carcinoembryonic antigens in relation to diagnosis, course of disease, and response to therapy. Appropriate reagents for the immunoglobulin and carcino-fetal antigen detections are prepared and evaluated. In addition, the Immunoglobulin Reference Center serves as a distribution outlet for provision of reference material for human serum immunoglobulin quantitation to qualified laboratories and in cooperation with the World Health Organization prepares and distributes international immunoglobulin reference serums. The Immunoglobulin Reference Center will also serve as a distribution point for carcino-fetal standard preparations when they are available. Finally, the Reference Center serves as a focus for the education of investigators and their technicians in the techniques and interpretations of various immunodiagnostic procedures.

Progress: CEA assays have been used to follow patients with metastatic disease during drug therapy. Preliminary data indicate that CEA fluctuates with, but does not precede, other measures of tumor load in patients with advanced cancer. Alphafetoprotein and human chronic gonadotrophin levels are also being determined on serums from patients with cancer.

Significance for Cancer Research (NCP Objective 5 Approach 4) A center with the capability of performing multiple immunodiagnostic tests will help to rapidly establish the usefulness of these tests in the diagnosis of cancer or as methods for monitoring patients during and after therapy.

Project Officer: W. D. Terry, M. D., M. Blaese, M.D., T. A. Waldmann, M.D.

Program: Immunodiagnosis

Site Visit Date:

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$470,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Cutaneous Hypersensitivity Antigen Reactions

Principal Investigator: Dr. Ariel Hollinshead
Name/Address: George Washington University
Performing: 1331 H Street, N.W.
Organization: Washington, D.C. 20005

Contract Number: NIH-NCI-G-69-2176

Starting Date: June 27, 1969

Expiration Date: June 26, 1973

Goal: To determine whether skin testing for delayed hypersensitivity reactions can be used to measure cellular immunity to tumor-associated antigens. To use the skin test technique as Approach: an assay for separation and purification of tumor antigens.

Extraction and purification of skin reactive antigens from carcinomas of breast, colon, stomach, lung and bladder; malignant melanoma; sarcomas; acute leukemias.

Perform skin tests on patients at various stages of disease, to determine relationship of reactivity to clinical course.

Progress: Extensive studies in colon carcinoma have shown that the skin reactive antigen is a carcinoembryonic antigen, distinct from the CEA of Gold. However, most CEA preparations from other laboratories and similar antigenic preparations have been shown to be contaminated with skin reactive antigen. Skin reactivity of patients with carcinomas of the lung and breast of melanoma patients have been found to be directed against tissue associated, as well as tumor associated, antigens. A blocking factor in soluble sonicates of tumor membrane extracts has been found, which inhibits reactivity to tumor antigens and not to bacterial antigens.

Significance for Cancer Research (NCP Objective 5 Approach 4)

A fundamental problem in tumor immunology is the demonstration of tumor specific antigens in human tumors. This contract provides an opportunity to study delayed skin reactions to antigens.

Project Officer: Dr. Ronald B. Herberman

Program: Immunology: Immunodiagnosis Site Visit Date: May 1969

Technical Review Group: Ad Hoc Review Committee

Relevance Review Group:

FY73 Funds: \$204,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Carcinoembryonic Antigen Program

Principal Investigator: Dr. Larry Nathanson
Name/Address: Tufts University School of Medicine
Performing: Boston, Massachusetts 02111
Organization:

Contract Number: NIH-NCI-G-71-2337

Starting Date: June 18, 1971

Expiration Date: September 1973

Goal: To explore the usefulness of the carcinoembryonic antigen in the diagnosis of carcinoma of the bowel and particularly in the early diagnosis of carcinoma of the bowel.

Approach: CEA determinations have been performed on the sera of patients with carcinoma of the gastrointestinal tract, of patients with other cancers, and with a variety of nonneoplastic diseases, and of normal individuals. A longitudinal study of populations at high risk of development of colorectal cancer were to be studied.

Progress: In the two years of the contract, the assays were set up and some sera tested. However, the rate of progress has been quite slow. The contract will, therefore, be phased out during the next contract year.

Significance for Cancer Research (NCP Objective 5 Approach 4)
CEA has been one of the more promising, though nonspecific, tests for diagnosis and follow-up of cancer patients.

Project Officer: William D. Terry, M.D., and Ronald B. Herberman, M.D.
Program: Immunology: Immunodiagnosis Site Visit Date: March 12, 1971
Technical Review Group: Tumor Immunology Review Group
Relevance Review Group: Tumor Immunology Advisory Group
FY73 Funds: \$37,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Purified Carcinoembryonic Antigen and Antisera

Principal Investigator: Dr. Charles W. Todd

Name/Address

Performing City of Hope National Medical Center
Organization: Duarte, California 91010

Contract Number: NIH-NCI-G-72-3877

Starting Date: June 1, 1972

Expiration Date: May 31, 1974

Goal: To provide purified carcinoembryonic antigen and antisera to carcinoembryonic antigen for use at the NCI Immunoglobulin Reference Center (Contract No. N01-CB-33875) in studies involving the immunodiagnosis of cancer. Purified CEA will also be prepared and supplied for collaborative studies involving the structure of this tumor associated antigen.

Approach: The contract will use presently available techniques to purify carcinoembryonic antigen. In addition, the contractor will investigate additional approaches for the preparation and isolation of these materials. Purified antigen will be used to immunize animals and produce antisera of appropriate specificity and strength for use in radioimmunoassay for diagnostic purposes. Purified CEA will be partially characterized from the chemical point of view, and where indicated, fragments will be prepared. These will be studied in collaboration with investigators of the Immunology Branch to determine the structure of the carcinoembryonic antigen protein.

Progress: Contractor has supplied antigen and antisera to a number of investigators and has conducted several training sessions for investigators interested in learning the technique. Continued production of CEA and supply of materials for collaborative efforts with NCI have been carried out. Studies substantiate the apparent chemical homogeneity of the protein portion of molecules carrying CEA activity.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Knowledge of the structure of CEA might make possible improved assays for detection of this carcinofetal antigen.

Project Officer: William D. Terry, M.D.

Program: Immunodiagnosis

Site Visit Date: April 28, 1972

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$141,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Blood Sample Collection Bank as Reservoir for
Evaluation of Cancer Detection Tests

Principal Investigator: Dr. Heinz Wahner
Name/Address Mayo Foundation
Performing 200 First Street, S.W.
Organization: Rochester, Minnesota 55901

Contract Number: NIH-NCI-G-72-3879

Starting Date: June 15, 1972

Expiration Date: June 14, 1973

Goal: To establish and maintain a bank of sera from patients
with cancer, with benign diseases and from normal individuals.

Approach: Make necessary serum samples available for evaluation
of immunodiagnostic tests for cancer.

Progress: A bank of specimens from over 1,000 patients has
been established. Coded sera have been distributed already
to a large number of investigators, for rapid evaluation of
potential immunodiagnostic assays.

Significance for Cancer Research (NCP Objective 5 Approach 4)

This serum bank will provide sera, in sufficiently large
quantities and with adequate controls, to efficiently and
rapidly evaluate potential immunodiagnostic tests for cancer.

Project Officer: Dr. Ronald B. Herberman

Program: Immunology: Immunodiagnosis Site Visit Date:

Technical Review Group: Tumor Immunology Review Group

Relevance Review Group: Tumor Immunology Advisory Group

FY73 Funds: \$55,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Carcinoembryonic Studies in Patients with Evaluable Lesions Given
Chemotherapy

Principal Investigator: Fred J. Ansfield, M. D.
Name/Address: The University of Wisconsin
Performing: 750 University Avenue
Organization: Madison, Wisconsin 53706

Contract Number: NIH-NCI-G-72-3881

Starting Date: June 20, 1972

Expiration Date: June 1974

Goal: To explore the usefulness of determining serum levels of carcino-embryonic antigen in evaluating the effect of chemotherapy on advanced malignancy of the colon and other organs.

Approach: The contractor will provide coded samples to the National Cancer Institute. The samples will be serums derived from patients with known types of malignancies and with measurable disease. Samples will be supplied prior to therapy and then at frequent intervals during the course of therapy. CEA assays will be performed at the NCI Immunoglobulin Reference Center (Contract PH 43-NCI-G-66-3).

Progress: Samples from patients are steadily being received at the NCI-IRC and these are presently being tested. Once sufficient numbers have been accumulated, the clinical and laboratory results will be compared. The accumulation of samples is nearing completion, and the contract will be terminated.

Significance for Cancer Research (NCP Objective 5 Approach 4)

CEA is one of the more promising tests for evaluating extent of malignant disease of the colon in relation to therapy.

Project Officer: William D. Terry, M. D.

Program: Immunodiagnosis

Site Visit Date:

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: None

CONTRACT RESEARCH SUMMARY

Title: Studies on the Application of New Immunological Techniques to the Specific Diagnosis and Therapy of Cancer

Principal Investigator: Dr. Michael Sela

Name/Address

Performing Weizmann Institute of Science

Organization: Rehovot, Israel

Contract Number: NIH-NCI-G-72-3890

Starting Date: June 26, 1972

Expiration Date: June 25, 1974

Goal: To adapt the bacteriophage plaque assay to the detection of carcinoembryonic antigen and alpha-fetoprotein.

Approach: The bacteriophage plaque assay, which has been extensively used in the contractor's laboratory, will be applied to detection of CEA and alpha-fetoprotein antigens. The contractor will purify these antigens by conventional approaches and explore various possibilities for attaching these materials to bacteriophage for the plaquing assay.

Progress: The contractor has developed a new technique for the specific binding of alpha-fetoprotein, using estradiol linked to sepharose. Apparently, specific antiserum has been produced. Attempts to use these reagents in the phage assay are under way. Preliminary studies of the phage assay using CEA have been unsuccessful.

Significance for Cancer Research (NCP Objective 5 Approach 4) More sensitive and stable assays for CEA and alpha-fetoprotein might improve the diagnostic usefulness of these carcinofetal antigens.

Project Officer: William D. Terry, M.D., Tibor Borsos, Sc.D.

Program: Immunodiagnosis

Site Visit Date: November 15-16, 1972

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$117,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: The Role of T and B Lymphocytes in Tumor Immunity

Principal Investigator: Dr. G. J. V. Nossal
Name/Address: The Walter and Eliza Hall Institute
Performing: of Medical Research
Organization: Royal Melbourne Hospital
Victoria, Australia

Contract Number: NIH-NCI-G-72-3889

Starting Date: June 30, 1972

Expiration Date: June 29, 1974

Goal:

The work of this project is to analyze the relative roles of T and B cell mediated immune responses in the rejection of tumors in mice.
Approach:

The approach will include an analysis of the mechanisms whereby tumor cells are killed and comparisons of various methods of inducing immune response against tumors. Plasma cell tumor systems will be utilized for at least some of the experiments, but fibrosarcomas, spontaneous reticulum cell sarcomas, paraffin oil induced reticulum cell sarcomas, and other experimental tumors will also be studied. All will be examined from the point of view of establishing optimum methods for producing T cell immunity. A portion of this work will require biophysical separation methods that will be applied to permit separate study of T and B lymphocytes. Techniques presently in hand, as well as new procedures that are under investigation will be applied to the separation of these cells and then the evaluation of the biologic function of the separated cells in tumor systems. The in vitro activation of T lymphocytes and an analysis of their killer activity will be undertaken. In addition, studies will be made of mechanisms for blocking killer cell activity. The final portion of the contract will deal with the mechanism of action of chemically defined adjuvants and their possible role in cancer immunology.

Progress: Two plasma cell tumors immunogenic in syngeneic mice have been selected from screening assays and established as cloned continuously cultured cells. After elimination of adherent cells, spleen cell suspensions have been found to be separable into B and T cell populations by electrophoresis. The tissue cultured plasma cell tumors have been found to be suitable targets for cell mediated immune assays in vitro and lymphocytes immunized in this way can inhibit tumor growth in vivo.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Understanding and manipulation of T and B cell populations are crucial to immunotherapy of cancer.

Project Officer: William D. Terry, M.D.

Program: Immunobiology Site Visit Date: Waived 1972

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$109,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Frozen Serum Samples for Carcinoembryonic Antigen Testing

Principal Investigator: Morris F. Collen, M. D.
Name/Address: Kaiser Foundation Research Institute
Performing: 508 16th Street
Organization: Oakland, California 94612

Contract Number: NIH-NCI-G-73-3852

Starting Date: August 15, 1972

Expiration Date: Nov. 14, 1972

Goal: To obtain sera from patients who have cancer, patients who developed cancer after the blood was drawn, and control subjects. These sera will be sent to the NCI for testing with CEA.

Approach: The contractor has collected sera during routine health checkups in their Northern California district between 1964 and 1969. Using their automated system of record keeping and capacity for computer search to correlate the stored sera with patient diagnoses, they will 1) search diagnoses from five sources: multiphasic examination follow-up physician visits, San Francisco outpatient diagnosis forms, Oakland Hospital tumor registry, San Francisco Hospital diagnosis index, and magnetic records of admissions to Kaiser Foundation Hospital in Northern California. 2) The medical record numbers of the cancer patients will be compared with the stored sera, and an appropriate number of sera (to be arranged in consultation with the Project Officer) will be removed from the freezer and shipped to NCI. 3) Specific information about each patient and controls will be provided to NCI in accordance with the contract proposal. 4) Sera from 200 normal persons of representative age, sex, and race will also be selected and sent from the group of stored sera. 5) Every effort will be made to fulfill the contract within a 3-month period.

The goals of this contract have been fulfilled.

Significance for Cancer Research (NCP Objective 5 Approach 4)

CEA analysis of human sera offers one of the more promising tests for certain cancers. This contract provides for standardization of this assay.

Project Officer: William D. Terry, M. D.

Program: Immunodiagnosis

Site Visit Date:

Technical Review Group: Tumor Immunology Scientific Review

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$10,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Control of Granulocyte and Macrophage Proliferation in Leukemia and Related Neoplasma

Principal Investigator: Donald Metcalf, M. D.
Name/Address: The Walter and Eliza Hall Institute
Performing: of Medical Research
Organization: Victoria 3050, Australia

Contract Number: N01-CB-33854

Starting Date: In negotiation

Expiration Date:

Goal: To examine the regulator substance known as colony stimulating factor (CSF) in relation to its formation, mechanism of action, and associations with cancer.

Approach: 1) Improve methods for producing the regulator substance Colony Stimulating Factor (CSF) and study its mode of action on target cells, both normal and neoplastic, 2) study the mechanisms by which antigens stimulate the formation of CSF, 3) evaluate the role of CSF in the regulation of granulopoiesis and monocyte formation in preleukemic and leukemic patients as well as normal humans and animals.

Progress: Newly approved contract.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Humoral factors, such as CSF, which regulate cells of the immune system are potentially powerful tools for immunotherapy.

Project Officer: William D. Terry, M. D.

Program: Immunobiology

Site Visit Date: Deferred

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$75,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Carcinoembryonic Antigen and Related Tumor-Specific Antigens in the Diagnosis of Cancer and as an Adjuvant in the Management of the Cancer Patient.

Principal Investigator: Dr. Tsann Ming Chu
Name/Address Roswell Park Memorial Institute
Performing 666 Elm Street
Organization: Buffalo, New York 14203

Contract Number: N01-CB-33859

Starting Date: In negotiation Expiration Date:

Goal: To evaluate the usefulness of carcinoembryonic antigen (CEA) assay in several varieties of human cancer. It will be evaluated for its capacity to 1) monitor recurrence of disease and 2) to measure the effectiveness of therapy.

Approach: This will be done by 1) determining a baseline value on all new patients who may fit into one of these categories, 2) evaluating CEA levels at 4 and 14 days after surgery (with some selected patients having more assays), 3) additional testing will be done at 30 and 60 days and every three months thereafter. For patients receiving radiation therapy, CEA testing will be done at two-week intervals during the therapy and at monthly intervals thereafter. Patients on chemotherapy will be tested at weekly intervals for ten weeks and then monthly.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 4)
CEA is one of the more promising assays for evaluating effects of therapy on certain cancers.

Project Officer: William D. Terry, M.D.
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Tumor Immunology Scientific Review Group
Relevance Review Group: Immunology Advisory Group
FY73 Funds: \$70,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Experimental Systems for Carcinoembryonic Antigen

Principal Investigator: Dr. David M. Goldenberg
Name/Address University of Kentucky
Performing Lexington, Kentucky
Organization:

Contract Number: N01-CB-33869

Starting Date: In negotiation

Expiration Date:

Goal: To produce carcinoembryonic antigen (CEA) which will then be provided to other investigators for analysis of its structure and to provide cell lines which produce CEA for use in studies of cellular hypersensitivity
Approach: in patients.

Will develop two human colon tumor lines maintained in hamster cheek pouches for the purpose of providing carcinoembryonic antigens in quantity to other investigators for analysis of the structure of the material. Will provide cell lines derived from the tumors carried in hamster cheek pouches for the purpose of studying cellular hypersensitivity in patients with various carcinomas.

Progress: New contract.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Structural analysis of CEA from various sources will improve understanding of its relationship to tumor origin and size.

Project Officer: William D. Terry, M. D.

Program: Immunodiagnosis

Site Visit Date:

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$50,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Isolation and Tissue Culture of Human Tumor Cells

Principal Investigator: Jørgen E. Fogh
Name/Address: Sloan Kettering Institute for Cancer Research
Performing: 145 Boston Post Road
Organization: Rye, New York 10580

Contract Number: In negotiation: Selection for RFP NIH-NCI-DCBD-73-19
Starting Date: Estimated June 1973 Expiration Date: June 1976
Goal: To provide human tumor materials needed for the performance of assays for cell-mediated immunity.

Approach: Tumor specimens from patients having the more common tumors, such as carcinomas of the colon, stomach, lung, breast, prostate, and bladder, will be obtained from local sources and utilized for the development of techniques suitable for the isolation of viable tumor cells in good yield separated from contaminating normal cells. Tissue culture techniques which favor the maintenance of high viability of tumor cells will be developed. Cells maintained in such culture will be characterized by morphology and as many other techniques as possible to document their tumor origin. Techniques will be developed for the slow freezing and retrieval of viable cells suitable for growth in tissue culture or for immediate use in immunologic assays.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 4)
Studies of cell-mediated immunity towards specific tumors as well as a general phenomenon in cancer patients is an urgently needed area of development in tumor immunology.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Advisory Group
FY73 Funds: \$127,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Procurement of Tumor Specimens from Cancer Patients

Principal Investigator: Eugene B. Rosenberg, M. D.

Name/Address

Performing University of Miami
Organization: Coral Gables, Florida 33124

Contract Number: In negotiation: Selection for RFP NIH-NCI-73-22

Starting Date: Estimated June 1973 Expiration Date: June 1976

Goal: To obtain specimens from human tumors of the colon, stomach, lung, breast, prostate, and bladder to be used for the development of immunodiagnostic assays for human cancer.

Approach: Surgical specimens of the above tumors to the extent of a minimum of 25 tumors of each type per year will be obtained under sterile conditions and in conjunction with normal control tissues from the same organ sites. Serum, EDTA plasma, and heparinized blood from some patients may also be required. In addition, a certain amount of dissection of tumor tissue and preparation of single cell suspensions, with appropriate packaging for shipping fresh and frozen specimens will be done.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

The application of known methodology to evaluate patient immune responsiveness requires the availability of human tumor specimens.

Project Officer: Ronald B. Herberman, M. D.

Program: Immunodiagnosis Site Visit Date:

Technical Review Group: Committee on Cancer Immunodiagnosis

Relevance Review Group: Tumor Immunology Advisory Group

FY73 Funds: \$85,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Collection of Serial Serum Samples from Cancer Patients

Principal Investigator: Eugene B. Rosenberg, M. D.

Name/Address

Performing

University of Miami

Organization:

Coral Gables, Florida 33124

Contract Number: In negotiation: Selection for RFP NIH-NCI-73-23

Starting Date: Estimated June 1973

Expiration Date: June 1976

Goal: Collection of serum specimens from patients with malignant diseases over a period of time to be used for the development and evaluation of potential immunodiagnostic tests for cancer.

Approach: Serum and plasma specimens will be obtained from patients with carcinomas of the colon, lung, breast, prostate, bladder, and other malignant diseases and from patients with benign diseases. A minimum of 25 patients per year of each category will be obtained. Specimens will be obtained before any therapy and at weekly intervals for one month and every two to three months thereafter.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

Readily available collections of sera obtained from patients during the course of therapy for their cancers are necessary for prompt evaluation of promising new immunodiagnostic techniques.

Project Officer: Ronald B. Herberman, M. D.

Program: Immunodiagnosis

Site Visit Date:

Technical Review Group: Committee on Cancer Immunodiagnosis

Relevance Review Group: Tumor Immunology Advisory Group

FY73 Funds: \$71,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Assays for Immune Depression in Cancer Patients

Principal Investigator: Richard L. Simmons, M.D., Jon R. Schmidtke, Ph.D.
Name/Address: University of Minnesota
Performing: Minneapolis, Minnesota 55455
Organization:

Contract Number: In negotiation: Selection for RFP NIH-NCI-DCBD-73-25
Starting Date: Estimated June 1973 Expiration Date: June 1976
Goal: To develop quantitative assays for the depression of various aspects of the immune response in human cancer patients and for following the clinical course of cancer patients.

Approach: Various parameters of the immune response will be evaluated in patients having carcinomas of the lung, breast, colon, prostate and bladder. These assays will be potentially applicable to large-scale use and will have been previously shown to be depressed in some cancer patients or to be depressed in animals with experimental tumors.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 1)
Assays for evaluation of the immune responsiveness of given individuals may prove to be highly important in understanding cancer patients' susceptibility to their tumors.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Advisory Group
FY73 Funds: \$130,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera From Populations With High Cancer Risk

Principal Investigator: John S. Najarian, M.D.
Name/Address: University of Minnesota
Performing: Minneapolis, Minnesota 55455
Organization:

Contract Number: In negotiation: Selection for RFP NIH-NCI-DCBD-73-27
Starting Date: Estimated June 1973 Expiration Date: June 1978
Goal: To collect serial serum specimens from populations at high risk of developing malignant disease for the purpose of developing and evaluating immunodiagnostic screening tests for human cancer.

Approach: Stable populations at high risk of developing the more common human cancers, such as colon, lung, breast, prostate, or bladder, will be followed on annual basis. This will include clinical evaluation and serum collection. The sera will be banked and records will be kept on the patients so that stored sera can be correlated with development of any particular cancer.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 1)
Early detection of cancer by means of appropriate diagnostic testing would permit treatment of cancer at the most favorable time.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Advisory Group
FY 73 Funds: \$75,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Collection of Sera From Populations with High Cancer Risk

Principal Investigator: Henry Altschuler, Ph.D.
Name/Address Philadelphia Geriatric Center
Performing 5301 Old York Road
Organization: Philadelphia Pennsylvania 19141

Contract Number: In negotiation: Selection for RFP NIH-NCI-DCBD-73-27
Starting Date: Estimated June 1973 Expiration Date: June 1978
Goal: To collect serial serum specimens from populations at high risk of developing malignant disease for the purpose of developing and evaluating immunodiagnostic screening tests for human cancer.

Approach: Stable populations at high risk of developing the more common human cancers, such as colon, lung, breast, prostate, or bladder, will be followed on annual basis. This will include clinical evaluation and serum collection. The sera will be banked and records will be kept on the patients so that stored sera can be correlated with development of any particular cancer.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 1)
Early detection of cancer by means of appropriate diagnostic testing would permit treatment of cancer at the most favorable time.

Project Officer: Ronald B. Herberman, M.D.
Program: Immunodiagnosis Site Visit Date:
Technical Review Group: Committee on Cancer Immunodiagnosis
Relevance Review Group: Tumor Immunology Advisory Group
FY73 Funds: \$45,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Conduct Bone Marrow Transplantation Studies

Principal Investigator: Dr. Clement Darrow
Name/Address: Litton-Bionetics, Inc.
Performing: 7315 Wisconsin Avenue
Organization: Bethesda, Maryland 20014

Contract Number: NIH-NCI-G-70-2103

Starting Date: March 9, 1970

Expiration Date: June 8, 1973

Goal: To establish a preclinical model for bone marrow transplantation in rhesus monkeys and to study and modify the graft versus host reaction (GVHR). A subsidiary goal is to study monkey transplantation antigens.

Approach: Rhesus monkeys are lethally radiated and then given histoincompatible bone marrow. Their subsequent course is followed clinically, hematologically, bacteriologically, virologically, chemically, and immunologically. Modification of GVHR is attempted by pretreating the recipient with antilymphocyte serum (ALS) and by destroying the allograft with cyclophosphamide and rescuing with frozen autologous marrow. Histocompatibility antigens are assayed on rhesus lymphocytes by the lymphocyte cytotoxicity test with defined antisera and by the mixed leukocyte reaction.

Progress: Takes of allogeneic bone marrow have been achieved with high frequencies (>90% of attempts). Acute GVHR has been reproducibly achieved in unmodified allograft recipients. It has been prevented by antilymphocyte serum pretreatment of the recipient and by the frozen autologous marrow rescue technique. Chronic GVHR remains a significant problem in the ALS treated allograft recipients, the average survival being about 40 days. Postgraft ALS has as yet been unsuccessful in prolonging survivals. A major histocompatibility system has been defined in the rhesus monkey and is designated RhL-A. We identify nine antigens of this system. Eight of these can be fitted into a hypothesis of two closely linked sets of multiple alleles.

Significance for Cancer Research (NCP Objective 6 Approach 1)
Bone marrow transplantation may allow more intensive cancer chemo- or radiotherapy than is presently possible. Transplantation of bone marrow may be important in cancer immunotherapy.

Project Officer: G. Nicholas Rogentine, Jr., M. D.

Program: Immunotherapy

Site Visit Date: April 5, 1973

Technical Review Group: Tumor Immunology Advisory Group

Relevance Review Group: Waived per Berlin's memo of Feb. 15, 1973

FY73 Funds: \$250,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Irradiate Experimental Animals

Principal Investigator: Staff
Name/Address: Armed Forces Radiobiological
Performing: Research Institute
Organization: Bethesda, Maryland 20014

Contract Number: NCI-GLC(70)-53

Starting Date: April 6, 1970

Expiration Date: June 30, 1973

Goal: To lethally radiate rhesus monkeys in preparation for bone marrow transplantation in rhesus monkeys and to study and modify the graft versus host reaction (GVHR), the major complication of this form of transplantation.

Approach: Rhesus monkeys receive 900 rads of X-radiation delivered by a Maxitron X-ray generator.

Progress: The contractor has satisfactorily radiated rhesus monkeys at our request.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Bone marrow transplantation may allow more intensive cancer chemotherapy than is presently available. Transplantation of allogeneic immune competent cells (bone marrow is one category) may be important in cancer immunotherapy.

Project Officer: G. Nicholas Rogentine, Jr., M. D.

Program: Immunotherapy

Site Visit Date:

Technical Review Group:

Relevance Review Group:

FY73 Funds: \$1,560

CONTRACT RESEARCH SUMMARY

Title: Measurement of Immunological Reactivity to Human Cancer

Principal Investigator: Dr. James McCoy
Name/Address: Litton Bionetics, Inc.
Performing: 5510 Nicholson Lane
Organization: Kensington, Maryland 20795

Contract Number: NIH-NCI-72-3227

Starting Date: 3/1/72

Expiration Date:

Goal: To study the immune responses of cancer patients to tumor associated antigens, and to correlate results of different assays with each other and with clinical course of disease.

Approach: A series of immunological tests have been set up to evaluate and monitor the general immune competence of patients and their response against tumor associated antigens. These assays include: lymphocyte stimulation by mitogens, recall antigens and tumor extracts; rosette assays for enumeration of T and B cells; leukocyte migration inhibition assays with recall antigens and tumor extracts; delayed hypersensitivity skin tests with tumor extracts; and cytotoxicity assays against tumor cells (microcytotoxicity assay and ^{125}I UDR cytotoxicity assay). Each of these assays will be performed serially on patients with various carcinomas, melanoma and lymphoma, and on benign disease controls and normal individuals.

Progress: During the first contract year, each of the above assays has been successfully developed. The lymphocyte stimulation and leukocyte migration inhibition assays have shown positive reactions with extracts from solid tumors. Cytotoxicity assays with short term cultures of tumors have given promising results, whereas tests with established cell lines have shown little specificity. The T and B cells in peripheral blood are being estimated, and this has already shown a decreased proportion of T cells in many cancer patients. All of these tests are being correlated with each other and with the clinical state of the patients.

Significance for Cancer Research (NCP Objective 5 Approach 4)

This contract will provide a comparison between several assays of cell-mediated immunity in tumor patients and provide information on the usefulness of these assays in immunodiagnosis of Project Officer: R.B. Herberman, M.D. cancer and in following the Program: Immunology: Immunotherapy clinical course of patients.
Technical Review Group: Ad Hoc Tumor Immunology Review Group
Relevance Review Group: Waived Site Visit Date: 1/72
FY73 Funds: \$219,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Studies on the Influence of Syngeneic Thymus Cells and of Thymic Humoral Factor Administration in the Control of Growth of Transplanted Tumors in Mice

Principal Investigator: Dr. N. Trainin

Name/Address

Performing Weizmann Institute of Science

Organization: Rehovot, Israel

Contract Number: NIH-NCI-G-72-3890

Starting Date: June 26, 1972

Expiration Date: June 25, 1973

Goal: To determine whether the induction of a "hyperthymic state" in normal animals will prevent or delay progressive proliferation of transplanted tumors.

Approach: Animals will be given large numbers of syngeneic thymus cells or crude thymic humoral factor. A wide range of concentrations of both of these materials will be tested for their ability to prevent proliferation of transplanted tumors in a prevention model or alter the course of transplanted tumors in a therapy model.

Progress: Baseline parameters of 3LL model system have been established. Removal of thymus and total irradiation caused increased numbers of lung metastases, and this could be revised by thymic reimplantation. Effect of crude THF and/or thymocytes on growth rate of 3LL tumors has been studied. In addition, studies of THF activity indicate that one of its effects is maturation of T cells.

Significance for Cancer Research (NCP Objective 6 Approach 1)

The role of the thymus, both cellular and possibly hormonal, on immune responses to tumors must be understood to permit appropriate manipulation of the immune system in tumor bearing hosts.

Project Officer: William D. Terry, M. D., John R. Wunderlich, M. D.

Program: Immunotherapy

Site Visit Date: Nov. 15-16, 1972

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$121,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Cancer: Studies on the Production In Vitro and Therapeutic Effects of Lymphocytes Specifically Sensitized Against Cancer Cell Antigens

Principal Investigator: Dr. Michael Feldman

Name/Address

Performing

Weizmann Institute of Science

Organization:

Rehovot, Israel

Contract Number: NIH-NCI-G-72-3890

Starting Date: June 26, 1972

Expiration Date: June 25, 1974

Goal: To study the ability of various selected lymphoid cell populations to cause retardation of tumor growth in a carcinogen-induced tumor model.

Approach: T and B cell populations will be sensitized against tumor and different T cell populations will be separately studied for their capacity to destroy tumor cells. Attempts will be made to remove potentially self-reactive lymphocytes before sensitization of these populations against syngeneic tumor cells. Various manipulations will be studied for effect on the growth of tumor cells in syngeneic systems.

Progress: Baseline parameters have been established for the 3LL tumor. Mouse lymphocytes have been sensitized in vitro and the effector cells (against allogeneic antigens) shown to be T cells. In vitro-sensitized T cells were found to recruit effector cells within the recipient. Cells having specificity for self antigens were detected. Cells autosenitized in vitro enhanced growth of 3LL cells in vivo.

Significance for Cancer Research (NCP Objective 6 Approach 1) Specific cell populations will have to be appropriately manipulated to take maximum advantage of the potentialities of immunotherapy.

Project Officer: William D. Terry, M.D., Herbert J. Rapp, Ph.D.

Program: Immunotherapy

Site Visit Date: November 15-16, 1972

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$138,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Cancer

Principal Investigator: Richard L. Simmons, M.D.
Name/Address: University of Minnesota
Performing: Minneapolis, Minnesota 55455
Organization:

Contract Number: NIH-NCI-G-72-3885

Starting Date: June 30, 1972

Expiration Date: June, 1974

Goal: The goal of this project is to test the usefulness of immunization with autochthonous, neuraminidase treated cells in the therapy of patients with widely disseminated human cancer not amenable to other techniques of treatment.

Approach: This will be done by (1) determining the immunologic competence of the patient, (2) determining the immunologic reactivity of the patient to his own and similar tumors, (3) instituting the trial of therapy with neuraminidase treated tumor cells derived from the patient, (4) redetermine the patient's immunologic competence at intervals during the course of treatment, (5) re-determine the patient's reactivity to his own tumor during the course of treatment, (6) utilize the criteria of objective remission used by cooperative chemotherapy groups in following the tumors in these patients, and (7) therapy will be discontinued whenever lack of response is demonstrated. Patients will be limited to those with melanoma, fibrosarcoma, osteogenic sarcoma, liposarcoma and neuroblastoma who have been demonstrated to have residual tumor that is not amenable to further treatment with either chemotherapy or surgery. In addition, these patients will have been demonstrated to have good immunologic responses.

Progress: Fifty-six patients were evaluated for inclusion in the study in the first 8 months. Nine died before therapy could be started. 48 patients have begun immunotherapy and 16 of these were still alive at the time of the report. Toxicity of BCG has been minimal and easily controlled. New protocols are now being developed for treatment of minimal residual disease.

Significance for Cancer Research (NCP Objective 6 Approach 4)

Modified autochthonous tumor cells have been shown to be promising immunotherapeutic agents in animals. This contract attempts to apply the animal work to human cancer patients.

Project Officer: William D. Terry, M.D. Site Visit Date: April 14, 1972
Program: Immunotherapy
Technical Review Group: Tumor Immunology Scientific Review Group
Relevance Review Group: Immunology Advisory Group
FY73 Funds: \$60,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Immunotherapeutical Trials with Human Tumors

Principal Investigator: Dr. Karl Erik Hellstrom
Name/Address: University of Washington
Performing: Seattle, Washington 98195
Organization:

Contract Number: NIH-NCI-G-72-3887

Starting Date: June 30, 1972 Expiration Date: June, 1974

Goal: The goal of this project is to evaluate the use of "deblocking" serum as well as immunization with autochthonous tumor cells as possible therapeutic approaches in the treatment of malignant melanoma. BCG may also be

Approach: Preliminary studies will be carried out to assure that neither of the immunotherapeutic maneuvers appears to have toxic affects on recipients. After this has been established, a double-blind immunotherapy trial will be established wherein patients will be assessed to determine whether or not their serum contains blocking antibodies in the colony inhibition assay. Patients with blocking antibodies will be randomized into a test group which will receive deblocking plasma and a control group which will receive normal plasma. Patients whose serum does not contain blocking factor will be randomized into a treatment group that will receive a course of immunotherapy involving active immunization with intradermal inoculations of tumor cells mixed with living BCG, and a control group which will receive no additional therapy. A variety of laboratory investigations will be carried out on these patients.

Progress: The plasma of approximately 50% of normal negro blood donors has been found to contain "deblocking" activity in cultures with melanoma patients tumor, serum, and lymphocytes. This provides a large enough source of plasma to treat a study group of melanoma patients. A phase I study has been completed and the approach is ready for randomized trial. BCG may be added to the therapeutic regimen. Neuroblastoma patients will not be studied because of insufficient numbers.

Significance for Cancer Research (NCP Objective 6 Approach 4)

This contract will provide for indepth laboratory analyses of patients immune response during immunotherapy and coordination of these responses with clinical course.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date: March 2
Technical Review Group: Tumor Immunology Scientific
Relevance Review Group: Immunology Advisory Group
FY73 Funds: \$163,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Graft Versus Tumor: A Model for the Treatment of Carcinoma, Leukemia and Lymphoma

Principal Investigator: Mortimer M. Bortin, M. D.
Name/Address Mount Sinai Medical Center
Performing Milwaukee, Wisconsin
Organization:

Contract Number: N01-CB-33853

Starting Date: In negotiation Expiration Date:

Goal: To evaluate adoptive immunotherapy using histoincompatible immunocompetent cells in the treatment of spontaneous leukemia-lymphoma in AKR mice.

Approach: Using spontaneous tumors in AKR mice: 1) combinations of chemotherapy and chemoradiotherapy will be evaluated for treatment of leukemia and for inducing immunosuppression. 2) H-2 incompatible immunocompetent cells will be administered to the immunosuppressed animals and graft versus host reaction will be monitored. 3) Chemotherapy and chemoradiotherapy plus antiserum against the H-2 incompatible cells plus immune H-2 compatible bone marrow will be administered for purposes of rescue from the graft versus host reaction.

Progress: Newly approved contract.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Development of a usable animal model using donor immunocompetent cells with subsequent "rescue" from graft versus host disease could be a highly effective method of immunotherapy in humans.

Project Officer: William D. Terry, M. D.

Program: Immunotherapy Site Visit Date:

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Immunology Advisory Group

FY73 Funds: \$60,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Neuraminidase Effectiveness in Tumor Immunotherapy and Mechanism of Its Effect

Principal Investigator: Dr. Gabriel J. Gasic
Name/Address: University of Pennsylvania
Performing: Philadelphia, Pennsylvania 19104
Organization:

Contract Number: N01-CB-33860

Starting Date: In negotiation

Expiration Date:

Goal:

To evaluate neuraminidase treated tumor cells in immunotherapy by establishing several animal tumor models and then examining the variables of dose, route of injection, etc., in these models. To examine the capacity of neuraminidase treatment in therapy of postsurgical metastatic cancer.

Approach: 1) test the effectiveness of neuraminidase treated tumor cells as immunotherapy in two spontaneous and three methylcholanthrene induced mouse tumors. 2) Use of model systems in which the neuraminidase treatment is effective the following parameters will be evaluated: dose of treated tumor cells, numbers of successive subcutaneous injections, route of treatment, and time of administration after tumor grafting. 3) Evaluation of the effectiveness of neuraminidase treated tumor cells in immunotherapy of metastatic tumor using metastatic tumor cells. 4) Examination of the effectiveness of neuraminidase modified cells in relation to the natural immunogenicity of certain target tumors.

Progress:

New contract

Significance for Cancer Research (NCP Objective 6 Approach 1)

Manipulation of autologous tumor with neuraminidase is one of the more promising areas of tumor immunotherapy. Results of this work will have direct relevance to man.

Project Officer:

William D. Terry, M.D.

Program: Immunotherapy

Site Visit Date:

Technical Review Group: Tumor Immunology Scientific Review Group

Relevance Review Group: Tumor Immunology Advisory Group

FY73 Funds: \$43,000

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy of Human Cancer

Principal Investigator: Jordan U. Gutterman, M.D.
Name/Address: University of Texas
Performing: M. D. Anderson Hospital
Organization: Houston, Texas 77025

Contract Number: In negotiation: Selection for RFP NIH-NCI-DCBD-73-15
Starting Date: Estimated June 1973 Expiration Date: June 1978
Goal: Evaluate BCG and neuraminidase treated autologous tumor cells as forms of immunotherapy in disseminated human cancer.

Approach: Patients with wide-spread involvement of a given cancer in relatively good condition but with a uniformly poor prognosis will be randomized with due regard for age, sex, general condition, and other variables determined by the particular tumor. Statistical considerations to insure evaluable results will enter into the randomization and the determination of patient numbers to be entered into protocols. Immunotherapy will be the sole experimental variable.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1 and 4)
Because of the wide-spread casual application of immunotherapy to human patients, it is important that well-designed prospective ethical clinical protocols be established as soon as possible.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Immunotherapy of Cancer
Relevance Review Group: Tumor Immunology Advisory Group
FY73 Funds: \$355,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Human Lung Cancer--Evaluation of BCG Therapy

Principal Investigator: Robert H. Waldman, M.D.
Name/Address: University of Florida
Performing: College of Medicine
Organization: Gainesville, Florida 32601

Contract Number: In negotiation: Selection for RFP NIH-NCI-DCBD-73-16
Starting Date: Estimated June 1973 Expiration Date: June 1977
Goal: Evaluate immunotherapy in cancer patients with a poor prognosis but in otherwise good health.

Approach: Newly diagnosed and previously untreated patients with oat cell and squamous cell carcinomas of the lung will be randomized with due regard for age, sex, and known variables for survival in the specific tumor. BCG will be administered by a schedule to be arranged with the Project Officer. All clinical studies necessary to establish the diagnosis and the extent of the cancer, as well as periodic documentation of the patient's level of health, tumor status, reaction to therapy, deviations from protocol, time to relapse, and survival time will be made. Certain specified immunologic tests will also be conducted.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1 and 4)
Because of the possibility that immunotherapy offers a distinct additive to other kinds of therapy, there is an urgent need to evaluate this therapy in patients who are not yet compromised by the extent of their disease.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Advisory Group
FY 73 Funds: \$99,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Human Melanoma: Evaluation of BCG Immunotherapy of Patients Without Detectable Disease After Removal of Tumor-Containing Lymph Nodes

Principal Investigator: Donald L. Morton, M.D.
Name/Address: University of California, Los Angeles
Performing: Los Angeles, California 90024
Organization:

Contract Number: In negotiation: Selection for RFP NIH-NCI-DCBD-73-17
Starting Date: Estimated June 1973 Expiration Date: June 1978
Goal: Conduct a suitably randomized study to evaluate promising preliminary evidence that BCG therapy is useful in melanoma.

Approach: Patients with a diagnosis of melanoma who have proven involvement of regional lymph nodes and whose disease has been totally removed as far as can be determined will be appropriately randomized into treatment groups with due regard for sex, age, and disease classification. BCG immunization will be performed with a strain, dose, and schedule to be determined in conjunction with the Project Officer. All clinical studies necessary to establish the diagnosis and document the patient's general health status, tumor status, toxicity of therapy, time to relapse, and survival time will be made. The patient's immunologic status will also be followed.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach and 4)
This is a direct extension of important preliminary observations regarding immunotherapy in human melanoma. This contract will permit a suitably controlled randomized clinical trial which should resolve a number of questions about this type of immunotherapy.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Advisory Group
FY73 Funds: \$200,000 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Cancer Immunotherapy: Animal Models for Treatment of Minimal
Residual Systemic Tumor

Principal Investigator: Gerald L. Bartlett, M.D., Ph.D.
Name/Address: M. S. Hershey Medical Center
Performing: The Pennsylvania State University
Organization: Hershey, Pennsylvania 17033

Contract Number: In negotiation: Selection for RFP NIH-NCI-DCBD-73-20
Starting Date: Estimated June 1973 Expiration Date: 1978 June
Goal: To develop animal models for the assessment of the usefulness and
parameters of administration of various types of immune stimulants as therapy
for cancer.

Approach: Animals models with a direct applicability to human cancer will be
studied for the effectiveness of various immunostimulants on their cancers.
The immunotherapy will be given after tumor load has been reduced by surgery,
radiation, or chemotherapy, and the tumor load will be quantifiable. Experi-
mental variables will include strain of organism, dose, schedule, and route
of administration.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1 & 4)
Evaluation of parameters of promising forms of immunotherapy in animals is
necessary for optimum utilization in humans.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Advisory Group
FY 73 Funds: \$146, 957 (estimated)

CONTRACT RESEARCH SUMMARY

Title: Immunotherapy: Development of Animal Models for Evaluation of Therapy with Neuraminidase Treated Tumor Cells

Principal Investigator: G. Mark Kollmorgen, Ph.D.
Name/Address Oklahoma Medical Research Foundation
Performing 825 Northeast 13th Street
Organization: Oklahoma City, Oklahoma 73104

Contract Number: In negotiation: Selection for RFP NIH-NCI-DCBD-73-21
Starting Date: Estimated June 1973 Expiration Date: June 1976
Goal: To explore the parameters of therapy with neuraminidase treated autologous or isogeneic tumor cells.

Approach: Using known successful animal models, parameters of immunotherapy with tumor cells, such as cell number, route and schedule of administration, methods and duration of cell storage, and use of tissue culture cells, will be studied. In addition potential new model systems for evaluation of this form of immunotherapy will be explored. Such systems will be required to have high applicability to human cancer; that is, therapy will be evaluated in the presence of established tumors and the tumors will be of relatively recent origin.

Progress: New Contract

Significance for Cancer Research (NCP Objective 6 Approach 1,4
Animal models for immunotherapy will not only contribute to the understanding of immunotherapy but also to the problems of diagnosis of the quantity of malignant disease and the evaluation of normal body mechanisms of defense against malignant disease.

Project Officer: William D. Terry, M.D.
Program: Immunotherapy Site Visit Date:
Technical Review Group: Committee on Cancer Immunotherapy
Relevance Review Group: Tumor Immunology Advisory Group
FY73 Funds: \$164,997 (estimated)

ANNUAL REPORT

THE BREAST CANCER PROGRAM COORDINATING BRANCH AND THE BREAST CANCER TASK FORCE PROGRAM July 1, 1972 - June 30, 1973

INTRODUCTION AND SUMMARY

This has been the first complete year given to the implementation of the Breast Cancer Task Force program as it was outlined, almost two years ago, in the "General Research Logic" charts. The result has been an increase in contract commitments from 21 at the end of June 1972 to 50 at the end of January 1973, with total estimated obligations increased from approximately 2.0 million (FY-1972) to 4.5 million (FY-1973). Much of the preparation for this large increment was accomplished the previous year. The four technical review committees of the Task Force took the lead in developing new projects and finding organizational resources to carry them out. The Breast Cancer Program Coordinating Branch, reorganized from the Endocrine Evaluation Branch, continued in the central role of coordinating the advisory committee activities with those of the Staff leading to the successful completion of contracts.

PROGRAM AIMS AND THE STRUCTURE OF SUPPORT

The "General Research Logic" of the Breast Cancer Task Force indicated that their major aims could be approached under four general headings: Diagnosis, Epidemiology, Experimental Biology and Treatment. Accordingly, four subcommittees of the Task Force were set up, and these in early 1973 were designated as chartered committees. At the same time the Endocrine Evaluation Branch was renamed the Breast Cancer Program Coordinating Branch and restructured to give each committee a corresponding section, the section head being executive secretary of the review committee. The technical review committees and the corresponding sections are as follows:

<u>Committee</u>	<u>Section in BCPCB</u>
Breast Cancer Diagnosis Committee	Diagnostic Projects Section
Breast Cancer Epidemiology Committee	Epidemiology Projects Section
Breast Cancer Experimental Biology Committee	Experimental Biology Projects Section
Breast Cancer Treatment Committee	Therapeutic Projects Section

The BCPCB, in parallel fashion, corresponds to the Task Force; it integrates the program as a whole (through the BCTF Steering Committee) with other programs of Division of Cancer Biology and Diagnosis and the National Cancer Institute.

BREAST CANCER DIAGNOSIS COMMITTEE PROGRAM

The Diagnosis Committee has been active in three areas: detection, immunology, and diagnosis-prognosis. It has developed contracts in these areas both through advertising (RFP's) and through review of unsolicited proposals.

In the detection area five contracts have been established. Three of these depend on ideas for new physical approaches to detection: an ultrasonic scanner, electronic mammography and a new X-ray source based on technetium-97m. Two collaborating investigators are utilizing breast fluid secretion for cytological evaluations and biochemical and virological studies. A small, short-term contract for the development of a biopsy tool has now been terminated, and the tool is being evaluated in a clinical setting.

In the immunology area there has been a threefold thrust to strengthen research in diagnosis. First, human study material is needed to assure a supply of breast cancer tissue for groups working on problems of cancer immunology, and one contract has been set up for this purpose. Secondly, since tumor material is seldom quantitatively adequate for all needs, long term human breast cancer cell lines need to be developed; and two contracts have been approved to support work along these lines. Finally, two contracts have been let for the purpose of developing immunological assays for human breast cancer-associated antigens, purification of antigens for human breast carcinomas, and evaluation of their specificity and usefulness in immunodiagnosis.

In the area of diagnosis-prognosis a program which aimed at the development of prognostic indices for primary and advanced breast cancer is being brought to completion. This program attempts to determine whether discriminants based on general clinical data, node involvement and hormone milieu of the patient can be used to identify the patient at high risk. It would thus help select appropriate means of treatment, perhaps during the "free period" between mastectomy and progression. Similarly, discriminants based on hormonal data are being sought to identify those patients who would respond favorably to adrenalectomy or hypophysectomy. The data accumulated in this program are now undergoing statistical evaluation.

BREAST CANCER EPIDEMIOLOGY COMMITTEE PROGRAM

A need exists to clarify the epidemiological aspects of breast cancer in order to identify population groups at different risks of incurring this disease. The expectation is that further study of such groups would lead to a delineation of specific etiologic relationships, or at the very least to development and institution of suitable control measures designed to minimize the risk.

With this objective in mind the Epidemiology Committee has directed its efforts in initiating research for the identification of those factors in population groups which might be correlated with variability in risk. The epidemiological association of some of these (age, race, sex, marital status, reproductive history and country of birth) to breast cancer incidence has already been explored to some extent; while the significance of others, such as chronic cystic mastopathies and other disease entities, is yet to be fully ascertained. The problem also remains to identify specific factors, such as

genetic, endocrinologic, immunologic, viral, chemical and physical, which could be associated with differences in risk and with the development of breast cancer.

With these priorities in mind, four RFP's were issued this year. One was aimed at clarifying the morphologic and epidemiologic characteristics of chronic cystic mastopathy and its relationship to breast cancer. Six proposals were received and considered in this category, and one will be funded. Another RFP concerning the role of diet and nutritional status on the incidence, pathogenesis and natural history of breast cancer led to the initiation of a study to determine to what extent differences in world-wide age specific breast cancer incidence curves could be accounted for in terms of simple anthropometric measures of obesity. The six proposals which were received in response to an RFP to investigate the role of exogenous estrogens or other hormones on the incidence, pathogenesis and natural history of breast cancer, and two received in response to an RFP to determine possible correlations between morphologic and epidemiologic characteristics of breast cancer did not prove satisfactory, but future synopses in this direction are planned.

In June 1972 the Epidemiology Committee sponsored a Workshop in Endocrinology to acquaint its members and interested parties with the state of the art of reproductive hormonal physiology, and to elicit ideas for projects clarifying the role of the hormonal milieu in the pathogenesis of mammary carcinoma. From this meeting an unsolicited proposal from one of the participants emerged, to study the dynamics of estriol metabolism in order to ascertain the physiological basis for repeated differences in urinary estriol levels between high and low risk breast cancer population groups.

Family and pedigree studies have long been a goal of the Epidemiology Program. A population-based study will be initiated in Iceland to determine whether there are hereditary factors which might identify a high risk cancer-prone group. The unique facilities available there including a nationwide cancer registry, a national roster and a family linkage file make this study particularly attractive. The Epidemiology Committee has also acted to establish a resource of some 80 breast cancer-prone families which will be available to collaborating investigators for a variety of clinical and laboratory studies, including searches for genetic markers and for other biochemical and physiological parameters.

BREAST CANCER EXPERIMENTAL BIOLOGY COMMITTEE PROGRAM

The program of the Experimental Biology Committee was initiated on the basis of five scientific areas in which specific research projects should be developed. One or more contracts have been initiated in each of the priority areas during the past year, and additional ones with related objectives have been sought through the advertising mechanism. The priority areas identified are as follows:

1. Hormone Action: Two problem areas identified were (a) the role of prolactin in mammary tumor growth; and (b) the biological significance of hormone dependency or independence of mammary tumors. For the first problem two contract projects were selected: one with the major objective of finding specific

receptor sites for prolactin in breast cancer; the other, with the goal of isolating and culturing in vitro lactotrophs from the adenohypophysis as a first step toward the understanding of prolactin production. Human and animal tissue will be studied in each of these projects. In the second problem area (hormone dependency) three contracts were funded. One contractor is attempting to determine whether a defect of the chromatin acidic proteins may cause the poor binding capacity for estrogens shown by non-hormonally dependent tumors. Another is extracting acidic proteins from the isolated chromatin, for acetylating, phosphorylating or methylating them, and determining whether this artificially induced defect may change the template activity of reconstituted chromatin as measured by the capacity to transcribe specific messages. The third contractor is studying the estrogen binding proteins of the cell cytosol to determine whether there is a defect of the 5S estrophile complex, the hormone "carrier," as evaluated by the action of this complex on RNA polymerase activity from nuclei of hormone dependent and independent tumors. Rat and human tissue are utilized for the first project and rat and mouse mammary tissue are employed for the last two studies.

2. Growth Regulation and Regression: Four contracts in this category were initiated. One is studying the involution of the normal mammary gland in vivo and the loss of differentiation of the lactating mammary cells in vitro. It has the objective of correlating differences in the regression process, between bovine glands, which are high in functional activity and low in neoplastic transformation, and rat glands which are the opposite. A second is studying the process of carcinogenesis, using organ cultures of rat mammary gland, to clarify the role of hormones in the process and the relationship between prolactin and estrogens in the onset and progression of mammary tumor growth. The third is studying the role of serum factors which stimulate DNA synthesis in organ cultures of mouse mammary gland. The fourth is studying the assembly of rough endoplasmic reticulum and the relationship between biosynthesis and degradation of individual membrane components using mouse mammary tissue.

3. Metastasis: Only one contract has been funded in this area, namely, one for evaluating the metastasizing capacity of rat mammary carcinomas when the membrane properties of the neoplastic cells and the immunological competence of the host are changed.

4. Role of Immunological Mechanisms in Development and Regression of Mammary Tumors: A single contract had been funded two years ago for studying the anti-tumor effect of MER (methanol extractable residue of BCG) in mouse mammary tumors. The project is basically one on immunotherapy and is more closely related to other programs of the Division of Cancer Biology and Diagnosis; therefore it will be terminated in the coming year.

5. Biological Characteristics of Normal and Abnormal Human Mammary Gland: Two problems were considered of major concern in this area: (a) development of long-term cultures of human mammary epithelium; and (b) characterization of precancerous lesions of the human mammary gland. For the first problem four contracts were selected, each employing a different culturing technique. Two are working with tissue culture, one with organ culture and the fourth is recording by time lapse photography the sequence of events occurring when an explant of human mammary gland or tumor is placed in culture media. Concerning

precancerous lesions, two projects of several years duration are under way, one a study of human glands, the other a study of mammary glands from the dog. Both projects have progressed to the point that they may be terminated in the coming year.

Another contract is funded which is of a service nature, namely, to maintain a tumor bank for the benefit of any contract program sponsored by the Breast Cancer Task Force program. Tumors of special biological characteristics, such as hormone responsiveness, are preserved.

New projects for which contractors were sought during recent months are classified under the five priority areas listed above. Two are directly related to hormonal dependency—one for studies of the role of cyclic-AMP, the other for studies on prostaglandins. A third project advertised is in the area of biological characteristics of normal and abnormal mammary glands, and specifically for studies of the mammary glands of pseudohermaphroditic hosts. The fourth is in the area of the regulation of growth and regression of mammary tissue, normal and neoplastic, and involves studies of the binding of effector molecules to mammary cell membranes and consequences of biological behavior. Lastly, a project related to metastatic studies was advertised, and sought work on the isolation and characterization of mammary epithelial cell membranes.

BREAST CANCER TREATMENT COMMITTEE PROGRAM

The Treatment program in brief has had three major aims: to initiate and extend research that would clarify the role of surgery and radiation in treating breast cancer, to promote and amplify studies involving combinations of treatments with oncolytic agents, surgery and hormonal modalities (including ablative surgery) and to support investigations tending to bring findings in the biology of breast cancer towards applications in the clinic.

Two of these objectives are served by support given to the National Surgical Adjuvant Breast Project, which has a study comparing total (simple) mastectomy and radical mastectomy, with and without radiation; and a study on the use of a chemotherapeutic agent, L-phenylalanine mustard as an adjuvant to surgery of primary breast cancer. The mastectomy study has entered approximately 750 patients from 32 institutions in a little over a year, and should finish entering patients in FY-1974; however it may be extended to include surgical options more limited than total mastectomy. The study with phenylalanine mustard is just getting started. Other studies utilizing 5-FU, Cytosin and Prednisone as surgical adjuvants are being considered. An RFP seeking a project combining chemotherapy and oophorectomy failed to bring forward a satisfactory proposal, and a new RFP to cover combinations of ablative therapy and drugs is being prepared. Animal models are being used in one contract laboratory to explore combinations of surgical intervention, standard oncolytic drugs, hormonal therapy and immunotherapy.

Development of the third objective is exemplified by the attempt to utilize determination of estrophilic proteins of tumors as a marker of endocrine dependency. A limited study at the University of Chicago (Dr. Elwood Jensen) has strongly suggested that breast tumors with high estrophile content frequently regress after the patient is adrenalectomized, while those lacking

estrophilic receptors seldom regress. There is widespread interest in applying the receptor measurement in a variety of clinical situations. An RFP for research and assays of estrophiles in a collaborative milieu brought many inquiries and at length seven worthwhile proposals, one or more of which will probably be funded.

Other instances of attempts to bring biological ideas closer to medical practice involves recent knowledge of cell kinetics and of tumor endocrinology. The Treatment Committee sponsored an RFP for new methodology in cell kinetics and another for a cell-kinetic approach to breast cancer that could be utilized in patients. Proposals in both categories are being considered. As to tumor endocrinology, studies by Meites, Pearson and others suggest that prolactin rather than estrogen may be the principal stimulating factor in breast tumor growth, and anti-prolactin drugs deter the growth of breast tumors. Many investigators have wished to incorporate prolactin determinations into their clinical trials as a first step in monitoring treatment with presumptive anti-prolactin drugs, and an unsolicited proposal for a laboratory capable of carrying out large numbers of determinations by immunoassay has just been accepted. These assays will be available to collaborating clinical groups which furnish satisfactory protocols for studies with a rationale involving prolactin.

THE FIRST ANNUAL WORKING CONFERENCE OF THE BREAST CANCER TASK FORCE

On February 5-7, 1973 the Task Force held its first annual working conference, at Williamsburg, Virginia. The 151 participants included representatives from all contracts, members of advisory groups, guests and Staff. The open session consisted of 49 presentations and lasted two days. A booklet containing abstracts of most of the papers was available to the participants, and since then has been revised and expanded for further distribution.

The closed session was attended by members of the Breast Cancer Task Force, NCI Staff engaged in this program, and a small number of other NCI advisors invited as guests. After a brief report on the program budget by the Chairman, the over-all program and the four specific program areas were reviewed and discussed critically. No substantial changes in basic plans (as outlined in the Flow Chart) were proposed, but there were suggestions which implied some expansion of activities—e.g., in the area of psychological adjustment of the patient to breast cancer and to treatment given after the onset of breast cancer.

FUTURE PROGRESS

As measured by contracts in force the program of the BCTF has doubled in the last year. The increment represents successful implementation of research plans long previously worked upon and agreed to as desiderata. An equally great leap cannot be expected again next year, both because money will be in relatively shorter supply (most contracts run more than a year) and because there is a smaller backlog of exploitable ideas on hand. Nevertheless each of the four technical committees does have high priority projects awaiting final approval on funds, and other less developed projects whose outlines and

desirability will become clear before the next year is out. New projects are therefore anticipated; at the same time the accumulation of approved projects will be greater than ever before and will require a large part of available resources and staff support.

CONTRACT RESEARCH SUMMARY

Title: An International Study on the Role of Overweight in Breast Cancer Epidemiology.

Principal Investigator: Dr. Frits de Waard

Name/Address State University of Utrecht, Kromme Nieuwe Gracht 29,

Performing Utrecht, The Netherlands

Organization:

Contract Number:

Starting Date:

Expiration Date:

Goal: To determine to what extent differences in world-wide age specific breast cancer incidence curves can be accounted for in terms of simple anthropometric measures of obesity.

Approach: Data will be collected on height and weight of all breast cancer and of control population samples in three areas of the Netherlands covered by population based cancer registries and the relationship between overweight, age and breast cancer risk will be studied.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5A Approach 1)

Project Officer: Dr. Bernice Radovich

Program: Breast Cancer Epidemiology

Site Visit Date: April 4, 1973

Technical Review Group: Breast Cancer Epidemiology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$24,500

CONTRACT RESEARCH SUMMARY

Title: Criteria for the Designation of Atypia in Chronic Cystic Mastopathy as Possible Premalignant Lesions.

Principal Investigator: Dankward Kodlin, M.D., M.P.H.

Name/Address Kaiser Foundation Research Institute, Department Medical Methods
Performing Research, 3779 Piedmont Avenue, Oakland, California 94611
Organization:

Contract Number:

Starting Date:

Expiration Date:

Goal: Studies will be formulated to investigate criteria for the designation of atypia in chronic cystic mastopathy as possible premalignant lesions.

Approach: From the Oakland Pathology Department's diagnostic files, stored on magnetic tape since 1963, 80 cancer cases with preceding benign lesions and 260 controls (benign lesions not followed by cancer) will be selected. About 1000 slides will be reread after they are coded to safeguard against bias. Histopathologic evaluation based on the Black-Chabon grading system plus one degree of atypia and subsequent development of mammary carcinoma.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5A Approach 1)

Project Officer: Dr. Bernice Radovich

Program: Breast Cancer Epidemiology

Site Visit Date: April 18, 1973

Technical Review Group: Breast Cancer Epidemiology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$49,696

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer and Estriol Dynamics

Principal Investigator: Christopher Longcope, M. D.

Name/Address Worcester Foundation for Experimental Biology, 222 Maple Avenue,
Performing Shrewsbury, Massachusetts 01545

Organization:

Contract Number:

Starting Date:

Expiration Date:

Goal: To determine the production and metabolic clearance of estriol in relation to high and low excretors in order to determine the physiological basis for repeated differences between high and low breast cancer-prone individuals.

Approach: This investigator plans to study the sources, blood production rates and urinary excretion dynamics of estriol in individuals with high and low E₃/E₂+E₁ ratios. Emphasis will be placed on the physiological mechanisms responsible for differences between subjects and within subjects at different times of their menstrual cycles.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5A Approach 1)

Project Officer: Dr. Bernice Radovich

Program: Breast Cancer Epidemiology Site Visit Date: March 23, 1973

Technical Review Group: Breast Cancer Epidemiology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$80,000

CONTRACT RESEARCH SUMMARY

Title: Breast Cancer Family Resources

Principal Investigator: Henry T. Lynch, M. D.

Name/Address Creighton University School of Medicine, Criss II Building,

Performing Room 312, Omaha, Nebraska 68131

Organization:

Contract Number:

Starting Date:

Expiration Date:

Goal: To establish as a research resource a pool of about 80 well-documented breast cancer-prone families to be used for medical genetic studies.

Approach: There will be continuous updating of genealogy and tumor history by personal interviews with patients and physicians and by telephone calls and letters. An augmentation from 50 (present members) to 80 families is planned. A mechanism for handling applications for access to the resource by qualified investigators (with separate funding) will be developed in consultation with Breast Cancer Task Force members and staff.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5A Approach 1)

Project Officer: Dr. Bernice Radovich

Program: Breast Cancer Epidemiology Site Visit Date: March 19, 1973

Technical Review Group: Breast Cancer Epidemiology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$95,120

CONTRACT RESEARCH SUMMARY

Title: A Study on Familiality of Breast Cancer to be conducted in Iceland

Principal Investigator: Dr. Hrafn Tulinius and Professor O. Bjarnason
Name/Address International Agency for Research on Cancer, 150 Cours Albert
Performing Thomas, 69008 Lyon, France
Organization:

Contract Number:

Starting Date:

Expiration Date:

Goal: To determine whether there are hereditary characteristics which might be of use in the identification of women having a high risk of developing breast cancer.

Approach: The unique facilities available in Iceland for the study will be utilized. These include 1) a nationwide cancer registry functioning since 1954 (about 800 cases), a computer based National Roster since 1952, and a family linkage file linking every individual alive after 1910 to his parents and children. If an excess risk is found, an effort will be made to correlate familial risk with recognized epidemiological risk factors for breast cancer.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5A Approach 1)

Project Officer: Dr. Bernice Radovich

Program: Breast Cancer Epidemiology Site Visit Date: Not required

Technical Review Group: Breast Cancer Epidemiology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$28,400

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Characterization of Animal Tumors in a Tumor Bank Facility

Principal Investigator: Arthur E. Bogden, Ph.D.
Name/Address: Mason Research Institute
Performing: 20 Harvard Street
Organization: Worcester, Massachusetts 01608

Contract Number: PH43-NCI-G-63-1146 (formerly PH43-63-1146)
Starting Date: June 28, 1963 Expiration Date: June 27, 1974
Goal: To maintain a bank of transplantable mammary tumors to be used by
BCTF and other selected investigators.

Approach: Mammary and related tumors preserved in frozen state.

- 1) Tissues preserved in Linde liquid nitrogen freezer following a tested procedure.
- 2) Submission of tumors by investigators able to furnish background information.
- 3) Protocol for testing A) viability and B) biologic characteristics of the preserved tissue. This includes: growth curve, peculiar effects on host organs, host survival time, effect on serum hormone levels, histology, cytogenetics, immunogenicity, response of tumor growth to ovariectomy.

Progress:

- 1) About 60 tumors have been shipped to 32 different investigators from the tumor bank in the past six months.
- 2) Sixty-two different types of tumors are presently stored in liquid nitrogen. Following the predetermined scheme 8 tumors were retransplanted, biological parameters are measured to define their major characteristics and then frozen again.
- 3) Transformation of R3230AC and 13762 rat mammary carcinomas into the ascites form has been initiated. The objective is to furnish investigators with neoplastic cell suspensions of mammary tumors, the latter metastasizing the former does not.

Significance for Cancer Research (NCP Objective ^{3,4,}5 Approach)

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.
Program: Breast Cancer Expt. Biology Site Visit Date: March 4, 1971
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY73 Funds: \$90,250

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Human Mammary Dysplasia Study

Principal Investigator: S. R. Wellings, M.D.
Name/Address: The Regents of the University of California
Performing: Davis, California 95616
Organization:

Contract Number: PH43-NCI-68-1461

Starting Date: June 28, 1968 Expiration Date: June 27, 1974

Goal: To characterize morphologically, functionally and etiologically precancerous lesions of human mammary gland.

Approach: Human mammary glands from autopsy and surgery are used for the following studies:

- 1) Subgross morphology of intact human breast using macrosections of dehydrated, defatted, colored, whole gland.
- 2) Electron microscopic (EM) analysis of mammary tissue.
- 3) Organ and cell culture to test response of mammary tissue to hormones.
- 4) Integration of morphological studies with viral, immunological and epidemiological programs.
- 5) Subgross analysis of 50 more mammary glands.
- 6) Termination of subgross atlas.
- 7) Larger effort in EM and culture studies of human precancerous lesion. Effort to be integrated with other programs.

Progress:

- 1) The atlas of human mammary gland pathology has been finished and is ready for publication.
- 2) The morphological classification of the mammary lesions is being utilized to detect these lesions in vivo without the need of tumor fixation. This work is being carried on with the objective of learning to transfer preneoplastic lesions of the human mammary gland in an appropriate culture medium.

Significance for Cancer Research (NCP Objective 5 Approach 4)

Results of these studies should aid in the diagnosis and classification of breast cancer lesions and provide data on precancerous lesions in the human breast.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Expt. Biology Site Visit Date: February 7, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$135,000

CONTRACT RESEARCH SUMMARY

Title: Programming and Data Processing Services

Principal Investigator: Francis L. Dugan, Jr.
Name/Address Automation Industries Inc., Vitro Laboratories Division
Performing 14000 Georgia Avenue
Organization: Silver Spring, Maryland 20910

Contract Number: NIH-NCI-G-69-58 (formerly NIH-69-58)

Starting Date: October 4, 1968 Expiration Date: July 27, 1973

Goal: To maintain an IBM system 360 data bank of hormonal bioassays and endocrine-related tumor data to be used by the BCTF or other interested investigators.

Approach: For every compound tested for its hormonal bioassays or effects against a battery of endocrine-related tumors to have separate magnetic tape storage files for:

- a) nomenclature using the IUPAC systematic name and/or CAS systematic, IUPAC trivial, CAS trivial, EEB trivial names.
- b) molecular formula using the HILL or NOPS system.
- c) source codes, i.e., the number assigned to the suppliers of the compounds.
- d) hormone bioassay data.

Information could be retrieved from any one of these files or incorporated onto one pre-designed print-out as related to a particular compound desired for review or publications.

Progress: All programs are written, tested, debugged and are operable. Some data referring to the more complicated types of testing such as combination, sequential, x-irradiation, etc., need to be entered into the master file. Program documentation is in the draft form and must be reviewed and finalized. System procedures and instructions for handling data input, editing and correcting exception reports are being written. These will be completed by the termination of this contract and then utilize the services of DGRT for maintenance, data retrievals and publications.

Significance for Cancer Research (NCP Objective 6 Approach 2)

Project Officer: Chester V. Piczak, B.S.
Program: Breast Cancer Exper. Biology Site Visit Date:
Technical Review Group: PRA Contract Review Committee
Relevance Review Group: Scientific Director, General Labs. and Clinics, NCI
FY73 Funds: \$19,497

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Study of Mammary Tumors in Dogs

Principal Investigator: Leslie J. Faulkin, Ph.D.
Name/Address: University of California
Performing: School of Veterinary Medicine
Organization: Davis, California 95616

Contract Number: PH43-NCI-G-69-33

Starting Date: August 21, 1969

Expiration Date: October 31, 1973

Goal: To follow the pathological history of the mammary gland in beagles, with particular emphasis on preneoplastic lesions.

Approach: Beagles of an AEC colony kept during their life span are utilized. Mammary glands at various periods of times and after various treatment.

- 1) Glands placed in Tellyesniczky, defatted, stained in H&E, dehydrated, cleared in toluene, stored in methyl salicylate. Nodules searched with dissecting microscope, counted, histologically classified.
- 2) Frequency and type of lesions correlated with age and effects of ovariectomy. Age 1-13 years:
- 3) Ligation of mammary ducts in virgin and mated subjects.
- 4) Transplant of suspected lesions in immunosuppressed mice and hamsters or in contralateral fat pad cleared before estrus was established.
- 5) Circulating levels of progestins, leutinizing hormone and prolactin under various conditions.
- 6) Examination of milk specimens in search for viruses.
- 7) Culture of mammary tissue.

Progress:

- 1) A classification of lesions based on 789 specimens has been completed and published.
- 2) Ovariectomy of 8-10 years old females did not change the pathology of mammary tumors over a period of 1 year after castration.
- 3) No correlation found between number of pups weaned and number of hyperplastic and neoplastic nodules.

Other experiments are in progress and some of them are expected to be terminated in 1972-73.

The next Progress Report is due in the summer of 1973.

Significance for Cancer Research (NCP Objective 3 Approach 1)

To determine ontogeny of precancerous lesions and to delineate interaction of precancerous lesions with the host.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Expt. Biology Site Visit Date: February 8, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$84,900

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Pre-Clinical Studies on Tumor Protective Activity of MER

Principal Investigator: David Weiss, Ph.D.

Name/Address The Hebrew University Hadassah Medical School, Jerusalem,
Performing Israel

Organization:

Contract Number: NIH-NCI-70-2208

Starting Date: June 29, 1970

Expiration Date: June 28, 1974

Goal:

To assay antitumor effect of the methanol extractable residue of tubercle bacilli (MER) in suitable mouse mammary tumors.

Approach: Mammary tumor virus (MTV) infected C_3H and MTV free BALB/cfc $_3H$ (fc $_3H$ infected with a variant of MTV of low oncogenicity) are used. Spontaneous tumors and isographs are removed surgically, either in toto or partially. Tail injections and subcutaneous retransplants are carried out to mimic metastasis. The types of studies done are: 1) Combined hyperimmunization with animal's own tumor (irradiated cells) plus more specific MER treatment. 2) Measurement of lymphocyte reactivity against target cells (A) blastogenic transformation of lymphoid cells kept in chambers and exposed to blastogenic cells. Reactant cells determined by ^3H thymidine incorporation and fluorescein-labeled antiserum specific to unique histocompatibility antigen. (B) Cr ^{51} release from target cells attached by lymphoid cells. 3) Response to dinitrochlorobenzene of guinea pigs sensitized by the hapten, with and without MER treatment. (Enhancement of delayed hypersensitivity).

Progress: See attached page.

Significance for Cancer Research (NCP Objective 6 Approach 4)

To investigate tumor-host relationships and to evaluate MER which offers a non-specific stimulant to the host's immune defenses.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: March 1, 1971

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$69,666

Principal Investigator: David Weiss, Ph.D.

PROGRESS: 1) No therapeutic effect of MER alone, reduced growth rate with MER + irradiation tumors used: Spontaneous mammary tumors and benzpyrene induced fibrosarcomas. 2) 5-FU + MER markedly retarded mammary tumor growth, methotrexate + MER prolonged survival time. 3) Irradiation + MER + 5-FU elicited marked synergistic retarding effect on mammary tumor growth. (Lack of precise statistical data to show that MER is improving the effect of any other agent). 4) Lymphoid cells from MER pretreated animals released more Cr^{51} from tumor cells than lymphoid cells for MER untreated mice. (technique not yet reliable) 5) MER treated guinea pigs developed stronger delayed hypersensitivity reactions to insufficiently sensitizing stimuli. 6) Synergistic retarding effect against lung metastases by MER + cyclophosphamide but little effect of either treatment alone. 7) In order to be effective as "pretreatment" (elevation of cellular immune responsiveness) MER has to be presented in "free" form not in an IFA mixture. "Pretreatment" may selectively favor cellular or humoral responsiveness to an antigen depending on conditions of treatment and strength of immunogenic stimulus. 8) MER produces local "cold abscesses" less extensive than living BCG - 6 X increase in popliteal lymphnode.

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Cellular Control in Mammary Involution and Dedifferentiation

Principal Investigator: Bruce L. Larson, Ph.D.
Name/Address: University of Illinois
Performing: Urbana, Illinois 61801
Organization:

Contract Number: NIH-NCI-G-72-3856

Starting Date: June 28, 1972

Expiration Date: June 27, 1974

Goal: To enhance our knowledge of dedifferentiation of neoplastic cells by studying the process of involution of the normal mammary gland in vivo and loss of differentiation of lactating mammary cells in vitro.

Approach: Using lactating mammary glands of cows and rats. Rat mammary carcinomas such as R3230AC which still have the capacity to produce small amounts of milk constituents.

- 1) Secretory mammary cells placed in vitro culture and production of milk constituents studies while cells are transformed from lactating into proliferating units.
- 2) Biological and biochemical characteristics of degenerating cells studied during gland involution induced by cessation of milk withdrawal.
- 3) Involuting rat mammary glands compared with those of cows to correlate differences in mechanism(s) of cell regression (cows have few mammary tumors as compared with rats. Is the involution process different?)

Progress:

- 1) Binding of oxytocin to myoepithelial cells and labeling secretory cells have been started. These are preliminary to an analysis of the effects of milk accumulation on cellular degeneration.
- 2) R3230AC rat tumor is cultivated and an evaluation of the specific milk proteins being synthesized is on the way.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Basically this is a study of the nature and modification of mammary tissue, normal and abnormal, which should form a basis for developing means to prevent conversions of cells to those capable of forming cancers.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Expt. Biology Site Visit Date: February 17, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$69,025

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Protein Nucleic Acid Interactions in Transcription Controls of Normal and Malignant Cells

Principal Investigator: Kenneth S. McCarty, Ph.D.
Name/Address: Duke University
Performing: Duke Medical Center
Organization: Durham, North Carolina 27710

Contract Number: NIH-NCI-G-72-3857

Starting Date: June 29, 1972

Expiration Date: June 28, 1974

Goal: To isolate, purify and fractionate nuclear acidic, non histone, proteins from mammary gland nuclei.

Approach: C₃H mammary glands and C₃H mammary adenocarcinoma, normal and leukemic lymphocytes and duck erythrocytes will be employed in the following studies.

- 1) Isolation of chromatin.
- 2) Extraction of acidic proteins. Published techniques to be perfected.
- 3) Binding capacity of extracted acidic proteins to DNA. Reconstitution of chromatin.
- 4) Capacity of reconstituted chromatin to transcribe specific messages.
- 5) Consequences of acetylation, phosphorylation and methylation on template activity of reconstituted chromatin.

Progress:

- 1) The acidic chromosomal proteins are phosphorylated as an immediate response to the addition of insulin to the culture medium.
- 2) A method for isolation of chromatin using saline trisodium citrate has been perfected.
- 3) A method for large scale preparation of nuclei has been devised. chromatin isolation from them was obtained, and fractionation of phosphorylated proteins from ammonium sulfate fractions was initiated.

Significance for Cancer Research (NCP Objective 3 Approach 3)

To increase knowledge of acidic protein properties is indispensable to improve understanding of hormone responsiveness of breast cancer.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Expt. Biology Site Visit Date:

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$49,180

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Diagnostic Characterization of Mammary Tumors

Principal Investigator: Joseph Leighton, M.D.

Name/Address The Medical College of Pennsylvania, 3300 Henry Avenue,

Performing Philadelphia, Pennsylvania 19129

Organization:

Contract Number: NIH-NCI-G-72-3858

Starting Date: June 28, 1972

Expiration Date: June 27, 1974

Goal: To develop a protocol for predicting the growth potential of human breast carcinomas.

Approach: Use embryonated eggs as recipients of transplanted human mammary carcinomas. Cultures of mammary tumor cells to be cultivated in meniscus gradients. Cultures of fibroblasts from normal and neoplastic tissues will be studied.

- 1) Chorionic allantoic membrane (CAM) transplants of human breast tumors and embryonal liver or kidney or lung, etc. The tumor is expected to grow in combination with the right tissue. CAM transplants of frozen-thawed human breast tumors will be attempted for clinical use in drug sensitivity tests. 2) Monolayers of human mammary carcinomas prepared using "spilling" or tissue dissociation techniques. Organoid patterns of meniscus gradient cultures related to clinical behaviors. 3) Morphological patterns and collagen production in cultures of fibroblasts from human carcinomas and normal gland samples at radical mastectomy. Variety of stroma in breast tumors derives from "altered" fibroblasts or influence of carcinoma cells on "normal" fibroblasts?

Progress:

- 1) Five human mammary carcinomas were transplanted in the chorionic allantoic membrane. In only 2 carcinomas cells survived. A papillary adenocarcinoma reproduced the same structure as in human biopsy. The 3 cases not growing were of the scirrhous type.
- 2) BT20 human mammary carcinoma line in CAM also grew poorly.
- 3) In miniscus-gradient cultures, BT20 line showed a separation of morphologically different cells. Whether this difference is related to a mixture of cells in the line or to different culture conditions is not clear at this time. Mucopolysaccharides appear able to protect cells from adverse culture conditions. The reasons for this protection are not clear.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Through the development of a method for maintaining human breast cancer in vitro it should be possible to achieve an accurate assessment of the probable course of the disease for each patient and the best course of treatment.

Project Officer: D. Jane Taylor, Ph.D.

Program: Breast Cancer Exper. Biology Site Visit Date: February 28, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$70,000

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Study the Role of Prolactin in Breast Cancer

Principal Investigator: Olof Pearson, M.D., and Jack Pensky, Ph.D.
Name/Address Case Western Reserve University
Performing 2040 Adelbert Road
Organization: Cleveland, Ohio 44106

Contract Number: NIH-NCI-G-72-3859

Starting Date: June 26, 1972

Expiration Date: June 25, 1974

Goal: To find specific receptor sites for prolactin in breast cancer tissue which might predict hormone responsiveness of individual breast tumors.

Approach: Human and animal mammary tumor tissue for the following studies:

- 1) Isolation and purification of human prolactin for radioimmunoassays.
- 2) Development of a specific immunoassay for human prolactin.
- 3) Isolation of membrane fraction from mammary tissue of pregnant mice. Incubation of this fraction with $^{125}\text{IOP2}$ + varying concentrations of unlabeled ovine prolactin. Presence of radioactivity on the cell membrane which can be displaced by unlabeled prolactin will be taken as evidence of specific binding. 7-12 DMBA tumors will also be used.
- 4) Measurement of prolactin levels in serum and other biological fluids in breast cancer patients.

Progress:

- 1) Dr. Pensky learned the Friesen technique for purifying human prolactin (Hpr). From 50 frozen pituitaries Hpr was isolated up to 72% pure by the Friesen assay. Radioiodination of the material was prepared but only 40% binding was obtained. Attempts to purify Hpr from human amniotic fluid are in progress.
- 2) The purified Hpr is used to immunize rabbits. An antiserum which binds ^{125}I Hpr to the extent of 50% at 1:2000 dilution is available and suitable to be used in radioimmunoassays of Hpr.
- 3) Serum prolactin levels are being measured in patients with metastatic breast cancer using homologous radioimmunoassay. Results are not yet sufficient for any conclusion.
- 4) First attempts to measure specific prolactin receptors in mammary tissue have had poor success.

Significance for Cancer Research (NCP Objective 5 Approach 6)

An approach directed toward the understanding of the role of the hypophysis in the growth of breast cancer. It is important to determine the presence of prolactin receptors and what their relations may be to estrogen receptors.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Expt. Biology Site Visit Date: February 18, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$72,314

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Documentation of Human Breast Cancer in Tissue Culture and the Recognition of Growth Patterns

Principal Investigator: Russell P. Sherwin, M.D.
Name/Address: University of Southern California
Performing: 2025 Zonal Avenue
Organization: Los Angeles, California 90033

Contract Number: NIH-NCI-G-72-3860

Starting Date: June 19, 1972

Expiration Date: June 18, 1974

Goal: 1) To compare human breast cancer in tissue form with cultured monolayers by direct observation and time-lapse film recordings. 2) To establish criteria for relating H&E classification of mammary tumors to in vivo and in vitro behavior.

Approach:

- 1) Tumor tissue, sliced thin enough to be transparent, is placed in culture media and the transformation of the original tissue into the culture monolayer is followed by time-lapse photos using phase contract microscopy.
- 2) Conventional histological examination of original tumor and standard in vitro culture preparations of cells thereof are carried out.
- 3) The pattern of outgrowth, the differential survival of various cell types, the intercellular relationships and the correlation between in vitro behavior and in vivo structure and clinical behavior will be studied.

Progress: Forty-seven carcinomas and 12 fibroadenomas of human breasts were studied in the first six months, 104 rolls of 16 mm film (100 ft.) were used to record cell outgrowth and cell interaction. Thirty of the 47 cases were observed in Rose chamber preparations. An average of 10-15 tube slips per case were prepared by May-Grünwald - Giemsa staining of the cover slip. These will be utilized for permanent records of culture outgrowths.

Significance for Cancer Research (NCP Objective 5 Approach 6)

The overall objective is to conduct laboratory studies of human breast cancer in vitro and to correlate the finding with clinical behavior of the disease which should provide new information on prognosis and possible etiology of the disease.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.
Program: Breast Cancer Exptl. Biology Site Visit Date: February 10, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$67,000

May 8, 1972

CONTRACT RESEARCH SUMMARY

Title: Hormonal Requirements of Normal and Abnormal Human Mammary Tissue in Organ Culture

Principal Investigator: Joel J. Elias, Ph.D.
Name/Address: The Regents of the University of California
Performing: San Francisco, California 94122
Organization:

Contract Number: NIH-NCI-G-72-3861

Starting Date: June 19, 1972

Expiration Date: June 18, 1974

Goal: 1) To find conditions to maintain growth of normal, dysplastic and neoplastic human mammary tissue in organ culture. 2) To see whether morphologic classification of human mammary lesions can be improved so as to be of prognostic value.

Approach: Human mammary tissue from biopsies, mastectomies and mammoplastics will be utilized for the following studies.

- 1) Electron microscopic, scanning electron microscopic and light microscopic preparations of normal and abnormal human mammary tissue to define histological, structural and surface characteristics.
- 2) Organ culture and eventually cell culture of material studied in (1) Analysis of conditions necessary for promoting growth.
- 3) Effects of hormones on organ cultures studied with EM, SEM and LM correlation with biological behavior in vivo.

Progress: Dysplastic and fibromatous tissue from 15 patients were cultivated in organ culture and exposed to different levels of insulin. Since insulin is indispensable for mouse mammary gland culture it was established that human mammary glands also used insulin but at a level higher than mouse gland. High levels of insulin produced squamous metaplasia of the dysplastic and fibroadenomatous epithelium. EM preparation of 3 dysplasias and 2 fibroadenomas have been completed and are now being studied. Improvement in the cultivation of scirrhous carcinomas have been obtained. Cultures from 11 patients showed that cell damage not the time of tissue preparation is more important than culture media in reducing culture "takes". The effect of embryonic mouse salivary mesenchyme on human cells of mammary carcinomas is being studied in relation to cellular differentiation.

Significance for Cancer Research (NCP Objective 4 Approach 1)

Efficient growth of normal and abnormal mammary tissue in vitro is an indispensable necessity for improving knowledge or cause, prevention and possibly treatment of the disease.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exptl. Biology Site Visit Date: February 9, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$69,964

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Biochemical Mechanism of Endocrine Induced Breast Cancer Regression

Principal Investigator: William L. McGuire, M.D.

Name/Address University of Texas Medical School at San Antonio, Department
Performing of Medicine, 7703 Floyd Curl Drive, San Antonio, Texas 78229
Organization:

Contract Number: NIH-NCI-G-72-3862

Starting Date: June 1, 1972

Expiration Date: May 31, 1974

Goal: To study the relationship between estrogen binding protein (EBP)
and hormone dependency of mammary tumors.

Approach: Human mammary carcinomas and metastasis thereof. Also primary, 7,12-DMBA induced, and transplantable R3230AC, MT/W9A, 13762E, 13762NF, 13762TP, 3M2N, rat mammary tumors will be studied in the following manner:

- 1) Quantitation of EBP in 8 rat mammary tumors and correlation to hormone dependency of each tumor.
- 2) Capacity of isolated chromatins to bind estradiol + EBP from exogenous cytosol. (This test is expected to show whether a "defect" of the chromatin acidic proteins may cause poor binding capacity and therefore hormone independence).
- 3) Search for correlation between loss of hormone dependency and chromatin acceptor activity during changes occurring in serial transplantation of rat tumors.
- 4) Quantitation of EBP absolute level in human mammary tumors and their metastasis. Correlation with their future behavior.

Progress: See attached page

Significance for Cancer Research (NCP Objective 5 Approach 6)

Assessment of estrogen binding sites for each human mammary tumor could become a routine clinical procedure for predicting therapy for the individual patient.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: February 24, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$127,000

- 1) The number of estrogen binding protein (EBP) sites has been quantitated in 65 primary and metastatic human breast tumors. A continuous spectrum from 0 to 612 f-moles/mg cytoplasmic protein was found. Specific ⁸⁵S binding was not detected in specimens with less than 9.0 f-moles EBP/mg cytoplasmic protein.
- 2) The interaction among receptor-estrogen-chromatin is influenced by temperature in the sense that nuclear accumulation of estrogen is influenced by temperature dependent receptor-estrogen binding to chromatin as well as temperature dependent penetration of estrogen into the cell.
- 3) No difference was found between target and non-target cells either in the ability of this nuclei to bind estrogen receptor or in the characteristics of the receptor after being released from binding.
- 4) MTW9 depends upon prolactin for growth, does not respond to ovariectomy and yet has clearly measurable estrogen receptor levels.

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Isolation of Prolactin Cells from Human and Rat Adenohypophysis; A New Approach to the Study of Control of Prolactin Secretion in Relation to Mammary Cancer

Principal Investigator: Wesley C. Hymer, Ph.D.

Name/Address The Pennsylvania State University, 207 Old Main Building,
Performing University Park, Pennsylvania 16802

Organization:

Contract Number: NIH-NCI-G-72-3863

Starting Date: June 28, 1972

Expiration Date: June 27, 1974

Goal: To isolate mammotrophs from human and rat adenohypophysis, to cultivate them and study their effects on mammary tissue, normal and neoplastic.

Approach:

The following materials will be used.

- 1) Human adenohypophysis from autopsy and surgical material.
- 2) Rat adenohypophysis from normal, lactating, estrogen treated and pituitary mamotrophic tumor bearing females.

The experiments will include:

- 1) Cell dissociation with trypsin spinner suspension flasks + Pasteur pipettes expulsion.
- 2) Sedimentation at 1G BSA gradient chamber NIH model.
- 3) Equilibrium density centrifugation.

Progress:

- 1) Sedimentation at unit gravity of freshly dissociated rat pituitary cells permits separation of a fraction containing over 60% mamotrophs estrogen administration produces hypertrophy of mamotrophs the same technique will be applied to rat mamotrophic tumors.
- 2) Prolactin content of mammotrophs has been determined when removed from normal, estrogen primed, lactating females. Immunoassayable prolactin within the sedimentation gradient parallels the distribution of mamotrophs estimated by light microscopy.
- 3) The technique of mammotroph isolation has been applied to human hypophysis. First on 4 glands obtained at autopsy then on 5 surgical specimens of breast cancer patients 20-30% of cells in the initial suspensions are mamotrophs their morphologic aspect is that of cells with increased prolactin synthesis.

Significance for Cancer Research (NCP Objective 3 Approach 3)

To further understand the role of prolactin in mammary tumor growth and to be able to interfere with tumor growth through manipulation of prolactin production one must use pure cultures of mammotrophs to analyze how the cells function.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: March 3, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$65,000

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Studies on the Prevention of Metastasis in Mammary Cancer

Principal Investigator: Untae Kim, M.D.
Name/Address: Health Research Inc., Roswell Park Division
Performing: 666 Elm Street
Organization: Buffalo, New York 14203

Contract Number: NIH-NCI-G-72-3864

Starting Date: June 15, 1972

Expiration Date: June 14, 1974

Goal: To assess the metastasizing capacity of rat mammary carcinomas when membrane properties of neoplastic cells and immunological competence of host are challenged.

Approach: Mammary carcinomas in W/FU rats that metastasize consistently will be utilized for the following:

- 1) Analysis of cell surface properties by (A) electron microscope (EM), (b) atpase and glycoprotein coat as shown by ruthenium-red binding, (c) fractionation of plasma membrane by hypotonic saline (DNA and G-6-pase of supernatant as controls) analysis of 5' nucleatidase and atpase activities and camp effort on properties of membrane preparation. (d) presence of surface antigens in the suspension fluid of cultures treated with membrane modifiers.
- 2) Response to PHA, CCA and PWM of lymphocytes from graft bearing metastasizing and non metastasizing tumors using H³ thymidine incorporation assay.
- 3) Presence of tumor cell surface antigens in host blood.
- 4) Collection of neoplastic cells thoracic duct, vein and arteries with the aim of understanding routes of metastases and ways of interferring with them using both approaches 1) and 2).

Progress:

- 1) Four new metastasizing mammary carcinomas have been isolated.
- 2) Certain enzymatic activities appear to be characteristic of metastasizing tumors, others of non-metastasizing ones. The difference in level of activity is several fold in one group as compared with the other.
- 3) Cell surface antigens of spontaneously metastasizing tumors cross-react with the goat antihuman carcino-embryonic-antigen (CEA). Circulating tumor cell surface antigens can be quantitated in these rats by human CEA.

Significance for Cancer Research (NCP Objective 4 Approach 3)

Immunological status of the host and metastasizing capacity of the tumor are two interdependent parameters and knowledge of the biological basis of this interdependence is essential for treatment of breast tumors.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Expt. Biology Site Visit Date: January 20, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$75,088

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Studies on Basic Biological Events in the Pathogenesis of
Mammary Cancer

Principal Investigator: Thomas L. Dao, M.D.
Name/Address: Health Research Inc., Roswell Park Division
Performing: 666 Elm Street
Organization: Buffalo, New York 14203

Contract Number: NIH-NCI-72-3865

Starting Date: June 15, 1972 Expiration Date: June 14, 1974

Goal: Determine the role of hormones and other host factors in the
pathogenesis of mammary dysplasia and neoplasia.

Approach:

1. In vitro experiments on carcinogenesis with organ cultures of rat mammary glands and analysis of hormonal effects on carcinogenesis.
2. Characterization of hyperplastic alveolar nodules (HAN) in the rat through transplantation, frequency in relation to neoplasia, in vitro behavior, hormone dependency.
3. Characterization of carcinogen induced (7,12-DMBA) tumors by estrogen receptors, content and histone acetylation of growing, regressing and regrowing specimens.
4. Definition of the relationship between prolactin and estrogen on mammary tumor growth through determination of hormone levels in blood, pituitary grafts and lesions of the medial eminence.

Progress:

- 1) A study on the relationship between occurrence of HAN and onset of mammary carcinomas has been completed. HAN were found much more numerous in the inguinal area but mammary carcinomas were equally distributed in all glands. HAN formation and tumor induction may be two separate phenomena.
- 2) A method for identifying in vivo HAN in Sprague-Dawley and Wistar-Furth rats has been developed. HAN can be removed under sterile conditions and cultured. The hormonal requirement of HAN is now being studied.

Significance for Cancer Research (NCP Objective 3 Approach 1)

Through studies of the nature of oncogenic cell transformations in carcinogenesis of mammary tissue develop means to prevent conversion of cells to those capable of forming cancer.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Expt. Biology Site Visit Date: January 20, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$79,875

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: The In Vitro Cell and Mammary Cancer (Phase I Feasibility Study)

Principal Investigator: William F. McLimans, Ph.D.

Name/Address Health Research Inc., Roswell Park Division, 666 Elm Street,

Performing Buffalo, New York 14203

Organization:

Contract Number: NIH-NCI-G-72-3866

Starting Date: June 15, 1972

Expiration Date: June 14, 1974

Goal: To achieve satisfactory long-term cultures of human mammary tissue.

Approach:

Utilizing human and animal mammary tissue, normal and abnormal.

- 1) Dissociation of tissue of origin into single cell suspensions; determination of optimal p₁₀² and p₂⁰ for mammary cells; determination of the optimal concentration of hormones in culture media under steady state conditions in collagen coated flasks.
- 2) Characterization of culture viability by morphology, rate of synthesis of β casein, β lactalbumin, and triglycerides.
- 3) EM analysis of the early events related to adaptation of cells in culture.

Progress:

- 1) Thirty-four specimens of human mammary tumors have been utilized for culturing. Infection has been a major draw back. pH, CO₂, and hormone requirements have been the parameters analyzed in short term cultures (10-14 days).
- 2) A tube rocker was developed to provide a thin film culture when the sample of cells was very small, as often occurs in biopsy material.
- 3) Procedures for cell dispersion from the tissue fragments are being optimized.
- 4) A calf serum dialysate is being tested to replace whole serum in the culture media. Attempts to grow mammary cells in serum-free media are proposed.

Significance for Cancer Research (NCP Objective 3 Approach 1)

The main objective of developing cell culture systems which retain native characteristics of the planted cells should aid in studies of the etiology of the disease which in turn should aid in prognostic and treatment studies.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: January 21, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$49,998

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Biochemical Nature of Hormone Dependency in Breast Cancer

Principal Investigator: Eugene DeSombre, Ph.D.

Name/Address University of Chicago, Ben May Laboratory for Cancer Research
Performing 950 E. 59th Street

Organization: Chicago, Illinois 60637

Contract Number: NIH-NCI-G-72,3873

Starting Date: June 1, 1972

Expiration Date: May 31, 1974

Goal: 1) To assess the effect of estrogen binding on the RNA polymerase activity of nuclei from hormone dependent and independent tumors. 2) To evaluate the biological meaning of the cytosol estrophile transformation from the native 4S unit to the 5S form.

Approach: Mammary tumors of Sprague-Dawley female rats will be induced with 7,12-Dimethylbenzanthracene for the following studies:

- 1) Separation of 4S and 5S cytosol estrophiles and determination of their binding capacity. Solubilization of nuclear receptors and their characterization by sedimentation analysis. Comparison between 8S and "salt stable 4S" estrophiles.
- 2) Use of temperature as a screening parameter for detecting binding capacity of nuclear fractions.
- 3) Analysis of effects on RNA-polymerase activity of the 5S estrophile complex isolated from hormone dependent and independent tumors exposed to estradiol.
- 4) Attempts to separate in the cytosol the factors which transform 4S into 5S component of the estrophile proteins.
- 5) Attempts to block tumor growth by depriving the nuclei of the 5S estrophile complex with estrogen which appears essential for RNA synthesis.

Progress:

- 1) Estradiol-17 β injected into castrated tumor bearing Sprague-Dawley females was utilized to study the relationship between ability of DMBA induced tumors to respond to hormonal manipulations and acquisition of insensitivity to hormones. A series of 224 animals with 669 tumors was studied and the over all phenomenon was outlined.
- 2) Nuclei from tumors which remain hormone dependent and from hormone independent tumor were isolated. The Parke-Davis-CI 628 antiestrogen compound is used to analyze its incorporation into these nuclei and in the cytosol. The competitive effects of CI 628 on the 4S to 5S transformation are being studied.

Significance for Cancer Research (NCP Objective 3 Approach 3)

The project looks at one aspect of the role of hormones in tumor growth. It is important to pinpoint the basic biological difference between hormone responsive and hormone non-responsive tumors. Could provide leads to treatment of the disease.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: January 21, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$99,982

May 8, 1973

CONTRACT RESEARCH SUMMARY

Title: Control of DNA Synthesis in the Mammary Gland

Principal Investigator: Frank E. Stockdale, M.D., Ph.D.
Name/Address: Leland Stanford Jr. University
Performing: Stanford, California 94305
Organization:

Contract Number: NIH-NCI-G-72-3875

Starting Date: June 1, 1972

Expiration Date: May 31, 1974

Goal: To determine the role of serum factor(s) in the stimulation of DNA synthesis in organ cultures of mammary gland epithelium of mice and mammary neoplasms of the same host.

Approach:

- 1) Tissue course of DNA synthesis stimulation by serum factor(s)
- 2) Dose response relationship between serum factor(s) to rate of DNA synthesis
- 3) Relationship between DNA synthesis and number of mitosis or length of generation time
- 4) Influence of serum factor(s) on estrogen sensitivity of mammary tissue
- 5) Nature of serum factor(s)
- 6) Kind of DNA synthesized during serum factor stimulation

Progress:

- 1) A 5% horse serum in cultures of mammary gland explants was found to produce four fold increase in the rate of thymidine incorporation and it is considered an optimal level for the study of stimulation of DNA synthesis.
- 2) Additive effect of insulin and horse serum was observed and it involves epithelial not adipose cells.
- 3) Porcine serum was found to be the most active - 50% $(\text{NH}_4)_2\text{SO}_4$ precipitated most of the active fraction. Most of the active fraction was retained by XM-100 and XM-300 filters. At 90°C - 63% of the activity was lost.

Significance for Cancer Research (NCP Objective 5 Approach 6)

Elucidating factors stimulating DNA synthesis in normal and abnormal mammary tissue should provide knowledge leading to the development of means to prevent conversion of cells to those capable of forming cancer.

Project Officer:

Program: Breast Cancer Expt. Biology Site Visit Date: April 10, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$73,798

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Study Breast Cancer Transplantability in Nude-Athymic Mice

Principal Investigator: Beppino C. Giovannella, Ph.D.

Name/Address The Stehlin Foundation, 834 Hermann Professional Building,

Performing Houston, Texas 77025

Organization:

Contract Number: N01-CB-33856

Starting Date: February 1, 1973

Expiration Date: January 31, 1974

Goal: To ascertain whether a Thymusless-nude strain of Swiss mice can be used as transplant-recipient of human tumors for a study of their growth potential and hormone responsiveness.

Approach: A colony of Thymusless-nude Swiss mice has been developed and the good health of the animals permits them to be used in long term experiments. Fifty human mammary carcinomas will be transplanted each year, each tumor to be transplanted into at least 3♀ and 1♂. Tumor fragments immediately after removal will be inserted subcut. into two fat pads and injected IP. Enrichment of the transplant with viable neoplastic cells will be attempted by pre-culturing the dissociated tissue - transplantation of some of these cases will be carried on. After transplantation the animals will be followed for 4 months. If the tumor grows it will be retransplanted for 3 manipulations as feasible. The transplanted tumor fragment will be characterized by a pathologist. The morphology and karyotype of the growing tumor will be compared with the original fragment.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 6)

The continued growth of human cancer cells of any origin in an animal would enable prognostic studies for treatment of the disease on an individual patient basis.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: October 27, 1972

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$90,000

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Studies on the Role of Prostaglandins in Mammary Gland Neoplasia

Principal Investigator: Dwight R. Robinson, M.D.

Name/Address Massachusetts General Hospital

Performing Boston, Massachusetts 02114

Organization:

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: To determine whether there is a difference in prostaglandin (PG) production in human mammary tissue, normal and neoplastic.

Approach: Production of PGE₂, PGF_{2α} and PGF_{1α} will be measured in the culture medium of human neoplastic breast tissue incubated for various times after surgical removal. Attempts will be made to obtain organ cultures of normal tissue derived from the same gland. Work in the investigator's laboratory has shown that colchicine and vinblastin enhanced PG synthesis in synovial tissue. The effect of antitumor agents will be tested on PG production by mammary tumors. Levels of cAMP will also be measured in the culture fluid. Radioimmunoassays will be used for PG and cAMP determinations.

The level of PG will be measured in blood serum of patients and attempts will also be made to measure PG in the efferent blood of the tumor. PG production and cAMP levels will be measured in the culture medium of two clonal tissues, one from rat mammary tumor and one from rat ovary. Both lines grow when stimulated by steroid and protein hormones. A correlation will be sought between PG production and initiation or arrest of growth. These lines and synovial fibroblasts, shown by the investigator to produce PG in culture, will constitute a reference point for the study on human material. Indomethacin has been shown to stop PG synthesis in all cell cultures tested in this laboratory; removal of the drug was followed by PG production. Blocking and resumption of PG production will be studied in human material and correlated with growth of the cultured tumor cells.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 1)

A study of a parameter of the biology of the cell (mammary) which should lead to new information never before studied.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: May 4, 1973

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$46,688 requested

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Studies on Role of Prostaglandins in Mammary Gland Neoplasia

Principal Investigator: Sumner Burstein, Ph.D.

Name/Address Worcester Foundation for Experimental Biology, Inc.

Performing 222 Maple Avenue

Organization: Shrewsbury, Massachusetts 01545

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: To measure the levels of prostaglandin (PG) biosynthesis in developing, adult and neoplastic mammary tissue.

Approach: Intercellular PG synthesis will be measured by incubation of the enzyme preparations with 8,11,14 eicosatrienoic and arachadonic acid under optimal conditions already established for preparations from seminal vesicles. The incubation mixture will be acidified, extracted with chloroform-methanol, treated with alkali, chromatographed in silica gel and counted. The enzyme preparation will consist of microsomal fractions from homogenates of the whole gland. Several parameters such as effects of glutathione, epinephrine or various inhibitors will be tested and compared with data from other tissues such as seminal vesicle which the author has studied. If microsomal fractions prove to be devoid of PG synthetase systems, whole homogenates or tissue slices will be used. Primary epithelial cell cultures will be used also. Dr. Fairbanks will supervise the operation. Tissue sources will be C₅₇BL, C₃H, C₃Hf, GR/A. The PG synthetase systems will be studied in developing, adult, pregnant, and lactating neoplastic tissue. If any difference is found, the effects in vivo and in vitro of PG inhibitors will be tested. Radioimmunoassays for PG metabolites are currently being developed. Monitoring of their levels in relation to PG synthetases will be pursued although detailed protocols cannot yet be drawn.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)

Develop the means to prevent transformation of normal cells to cells capable of forming cancer through investigations of cellular and organismal modifiers of the transformation and promotion process.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: May 3, 1973

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$47,650 requested

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Studies of the Biochemical Means by which Effector Molecules Bind to Mammary Cell Surfaces and Influence Cellular Biochemistry

Principal Investigator: Steven D. Douglas, M.D.

Name/Address Mount Sinai School of Medicine

Performing Fifth Avenue and 100th Street

Organization: New York, New York 10029

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: To study the interaction between lymphocytes and normal or neoplastic mammary cells.

Approach: Lymphocytes from heparinized peripheral blood will be tested against fresh mammary tissue, cultured mammary cells and their sonicated extracts. Lymphocyte mediated immunity will be assessed by lymphocyte transformation, leukocyte migration inhibition, elaboration of macrophages inhibition factor and blastogenic factor. Direct cytotoxic effects of activated lymphocytes will be assayed by the method of Cerottini. Sensitivity of mammary cells to the cytotoxic factor will be tested by a modification of the Granger technique. The cytotoxic factor will be produced by a human lymphoid cell line and column-purified. The effect of this factor on DNA, RNA and protein synthesis will be tested in autologous cell systems. About 100 patients are to be tested. Various cell types in culture will be evaluated by EM in relation to organelle development and to the effect produced by lymphocytes or effector molecules. Attempts will be made to characterize biochemically the binding site of effector molecules and possibly to elucidate the nature of the effector molecule-receptor site reaction. Parameters measured in normal and neoplastic breast tissue will include: ^3H thymidine uptake, RNA polymerase activity, molecular species of RNA produced, ribonucleoprotein characterization by ultracentrifugation, uptake of labeled amino acids into acid precipitable proteins, turnover of phospholipids and levels of some lysosomal enzymes.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)

Study cell surface and cell membranes in obtaining new information as to means of preventing the transformation of normal cell to neoplastic ones.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: March 30, 1973

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$99,612 requested.

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Studies of the Biochemical Means by which Effector Molecules Bind to Mammary Cell Surfaces and the Manner in which they Influence Cellular Biochemistry

Principal Investigator: Kurt E. Ebner

Name/Address Oklahoma State University, Department of Biochemistry

Performing Stillwater, Oklahoma 74074

Organization:

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: To study composition and biochemical properties of normal and neoplastic mammary cell plasma membranes.

Approach: Single cell suspensions will be obtained from normal and lactating mammary gland and from mammary tumors (R3230AC of rat and C₃H mouse adenocarcinomas + MTV). The methods of Howard and Pesch and that of Prop are routinely used in the laboratory. Separation of cell types will be attempted using differential centrifugation and also a specific activity column. It is proposed to synthesize such a column by binding prolactin chemically to a partially hydrolyzed nylon support. Assessment of the damage inflicted on the cells by the isolation will involve comparison of glucosamine ¹⁴C, furan ¹⁴C and leucine ³H uptake into membrane protein of freshly isolated and incubated cells which have "repaired" their damaged membranes. Similarities and differences of surface structures between tumor and mammary cell membranes will be evaluated by the binding of ¹²⁵I CON. A and the lutins. A computer program to analyze the data of binding interactions is presently available in the lab. Isolation of cell membranes will be achieved by sucrose density centrifugation of cells broken by homogenization or N₂ cavitation with or without treatment with membrane-stabilizing agents. Distribution between two polymeric phases, affinity chromatography, affinity density perturbation and isoelectro focusing will be used to purify membrane preparations. Purity will be assessed (1) by EM and phase-contrast microscopy (2) by sialic acid, cholesterol protein and phospholipid analysis and (3) by Na⁺, K⁺ ATPase and 5 nucleotidase. Membrane glycoproteins will be isolated, this structure studied with regard to numbers and size of carbohydrate units, composition of units and glycopeptide linkages. Quantitative binding of prolactin to cell membranes using Cuatrecasas' procedure.

Progress: New contract

Significance for Cancer Research (NCP Objective 3 Approach 5)

Study of cell surfaces and cell membranes in order to obtain new information which would be useful in developing means to prevent transformation of normal cells to neoplastic ones.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: March 8, 1973

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$122,532 requested, reduction to be negotiated

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Studies of the Biochemical Means by which Effector Molecules Bind to Mammary Cell Surfaces and the Manner in which they Influence Cellular Biochemistry

Principal Investigator: Zoltan J. Lucas, M.D.

Name/Address Leland Stanford Jr. University

Performing Stanford, California 94305

Organization:

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: To study the modification of the host's response by mammary tumors by focusing on tumor angiogenesis factor (TAF) and specific and nonspecific inhibitors of cellular immunity (ICI).

Approach: Production of TAF will be attempted in vitro using multilayer and nodular tissue culture techniques of existing mammary tumor lines. An assay system for TAF will be developed based on the possible stimulation by TAF of monolayers of endothelial cells. Cultures of rat endothelial cells can now be maintained for only a few days and the methodology must be improved. The "air-sac" or "corneal neovascularization" assays for TAF will be used only for standardization. Attempts will be made to stabilize TAF in solution as well as to prepare an anti-TAF serum. The technique applied by Lucas to prepare anti-human lymphotosin will be utilized here. The immune response to tumor antigens is currently being measured by Lucas utilizing 6 assays (cytotoxic antibodies, blocking antibodies, lymphocyte-dependent antibodies, autotonic cells, products of antigen activated lymphoid cells and "activated" macrophages). A determination of whether nonspecific or specific inhibitors of cellular immunity are directly or indirectly related to the presence of human mammary tumors will be attempted.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 1)

To determine whether nonspecific or specific inhibitors of cellular immunity are directly or indirectly related to human mammary tumors.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: March 12, 1973

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$90,346 requested

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Isolation and Characterization of Mammary Epithelial Cell Plasma Membrane

Principal Investigator: Grant Fairbanks, Ph.D.
Name/Address: Worcester Foundation for Experimental Biology, Inc.
Performing: 222 Maple Street
Organization: Worcester, Massachusetts 01545

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: To investigate changes of the cell surface of mouse mammary epithelium undergoing hormone-dependent differentiation in vivo and short term primary monolayer cultures.

Approach: Isolation of mammary cell membranes will be attempted by applying affinity chromatography methods. Synthesis of three reagents will be undertaken, (1) sulfonic acid diazonium salt with an attached isomaltose chain, (2) an iminoester designed to couple pyridonal phosphate to the cell surface, (3) a sulfonate group will be introduced on activated polyacrylamide beads to provoke steric hindrance of the coupling of proteins. Cell suspensions from mammary gland of CD-1 mice will be prepared following McGrath's procedure. A more sophisticated method of cell separation may be necessary to obtain re-producible fractions of the parenchymal cell population. Sedimentation in dextran-BSA or polymer phase partitioning will be tried. Reagents (1) and (2) are expected to react spontaneously with amine groups of the cell surface in phosphate buffered saline pH8. Reagent (3) will require a brief incubation at pH 5-6 to form Schiff's bases with amines of the membrane. Labeling of the cytoplasmic protein fraction will yield an estimate of the permeability of the cell membranes to the reagents. Labeled cells will be disrupted by nitrogen cavitation and subjected to differential centrifugation and isopycnic banding. Recovery of 5'-nucleotidase and Mg^{++} dependent, $Na^{+} + K^{+}$ stimulated ATPase will be evaluated. The crude membrane fractions will be selectively absorbed by exposing them to the appropriate ligand. Ligand for reagent (1) is antidextran antibodies, for reagent (2) it is pig heart aspartate amino transferase acyl azide derivative of porous polyacrylamide beads. Isolated membranes will be characterized by phase contrast and EM, polyacrylamide gel electrophoresis, chemical and enzymatic composition and physiochemical studies of fragment heterogeneity. The analytical procedures will be carried on with the goal to establish whether a reorganization of the cell surface accompanies neoplastic transformation.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 5
Develop method of isolation of cell surfaces and cell membranes.

Project Officer:

Program: Breast Cancer Exper. Biology Site Visit Date:

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$46,875 estimated

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Studies on the Role of 3',5'-Cyclic Adenosine Monophosphate and Related Enzyme Systems in Mammary Gland Neoplasia

Principal Investigator: David Maudsley, Ph.D.

Name/Address Worcester Foundation for Experimental Biology, Inc.

Performing 222 Maple Avenue

Organization: Shrewsbury, Massachusetts 01545

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: To study the role of cyclic adenosine 3',5'-monophosphate (cAMP) in the developing mouse mammary gland and to establish whether the incidence of mammary tumors alters cAMP metabolism.

Approach: Separation of epithelial from adipose cells will be obtained using collagenase (or other published procedures) in glands of virgin, pregnant and lactating CD-1, C57 BL, GR, C3H, C3Hf mice. Subcellular fractionation procedures will be applied to the localization of adenylyl cyclase, cAMP-receptor protein and phosphodiesterase and their relative amount determined. cAMP will be determined with the protein binding assay using bovine adrenal protein, and free from hormonal nucleotides will be separated by BSA-coated charcoal. Phosphodiesterase and adenylyl cyclase determinations will be based on the specificity of cAMP binding protein following a procedure developed in Maudsley's lab. Formation of cAMP in intact cells will be studied by preincubating tissue slices or cells with prelabeled precursors. Isolation of labeled cAMP and related nucleotides will follow using proper chromatography (Rosen and Rosen). The method is current in Maudsley's lab. cAMP metabolism is regarded as an integrated unit, the major components of which are a receptor-adenylyl cyclase complex, cAMP itself, a cAMP receptor protein and phosphodiesterase. The individual components will be studied in isolation and an attempt to establish their relative significance within the intact cell and eventually in the whole gland will be made.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)

Develop the means to prevent transformation of normal cells through investigation of cellular and organismal modifiers of the transformation and promotion process.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: May 3, 1973

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$72,300 requested

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Studies on the Role of 3',5'-Cyclic Adenosine Monophosphate and Related Enzyme Systems in Mammary Gland Neoplasia

Principal Investigator: David F. Scott, Ph.D.

Name/Address Medical College of Georgia

Performing Augusta, Georgia 30902

Organization:

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: Activity levels of 3',5'-cyclic adenosine monophosphate and the enzymes responsible for its formation and degradation will be correlated to growth rate of mammary tumors to see whether any meaningful generalization can be made.

Approach: Adipose, ductal and alveolar cells of normal and developing mammary gland and neoplastic cells of mammary tumors will be isolated. The activity of adenylate cyclase and phosphodiesterase will be measured in homogenates of whole glands, in isolated cells, in mammary gland explants and in cultures of each cell type. The hormonal regulation of cAMP levels will be studied using the adenosine prelabeling procedures as developed by Kuo and by Schultz and Daly. C₃H and A mice will be used first, human tissue later. Mice with normal hyperplastic nodules and carcinomas will be obtained under the supervision of Dr. A.G. Liebelt. Cell isolation and cultures will be carried on under the supervision of Dr. E. Howard. Growth rate and cAMP levels will be compared in tumors and in normal cells. The main objective is to understand the mechanism(s) by which cAMP levels are controlled in mammary tissues and isolated cell preparations, both adipose and epithelial.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)

Study of enzymes systems which may contribute important information on the cause of the disease and to possible methods of treatment and prevention.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: April 20, 1973

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$85,874 requested

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Studies of Isoenzymes in Normal, Pre-Neoplastic and Neoplastic Breast Tissues as an Approach to Define Unique Properties of Neoplasia

Principal Investigator: Russell Hilf, Ph.D.
Name/Address Department of Biochemistry and Division of Oncology
Performing University of Rochester School of Medicine and Dentistry
Organization: Rochester, New York 14642

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: To utilize glucose 6P-dehydrogenase and lactate dehydrogenase isoenzyme patterns to characterize normal, preneoplastic and neoplastic mouse mammary tissue.

Approach: The isoenzyme patterns will be examined in normal breast at various stages of development, during pregnancy, lactation, after parturition, in preneoplastic and neoplastic conditions. Preliminary experiments showed a sharp increase in the quantities of G6PD1 in tumors versus virginal and lactating gland. The isoenzymes for mammary tumors will be purified and antisera will be produced. A radioimmuno assay will be developed for use as a discriminant assay on the assumption that differences in isoenzyme quantities characterize neoplastic and preneoplastic tissues. These studies will be carried on in parallel with G6PD and LDH isoenzymes profiles in normal and neoplastic human tissues now supported by grants. The rodent work is done to permit an analysis of the effects of exogenous hormones and the ablation of endocrine organs on the isoenzyme profile.

Progress: New Contract

Significance for Cancer Research (NCP Objective 4 Approach 1)

Investigate the biology of cells already capable of forming cancer to determine the relationship of their growth and interaction with the host response.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.
Program: Breast Cancer Exper. Biology Site Visit Date:
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY73 Funds: \$35,108 requested

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Identification of Cell Types in Cultures of Normal and Neoplastic Breast Tissues

Principal Investigator: Samuel Abraham, Ph.D.

Name/Address Children's Hospital Medical Center of Northern California

Performing Fifty-first and Grove Streets

Organization: Oakland, California 94609

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: To identify cells - markers in normal and neoplastic breast cultures of mouse, dog and human origin.

Approach: Antisera will be prepared using whole cells, cell membrane and microsomal preparations as antigens. Adipose and fibroblast cells will be obtained from the cleared fat pad, thus separation for the epithelial cells. Methods for separating parenchymal from stromal cells in the mammary gland have been developed in Abraham's lab. in the past. Myoepithelial cells will be identified using an anti-myosin serum prepared from muscle myosin. Antibodies will be conjugated with peroxidase and reacted with a different color producing substrates. Attempts will be made to characterize the nature of the cultivated cells by the color assumed by each cell type. Fibroblasts will be identified by the proline hydroxylase and lysyl oxidase activities. Lactose synthetase B protein will be purified and an antiserum will be produced and used to detect the presence of the protein in epithelial cells. The multienzyme complex fatty acid synthetase has been isolated in Abraham's lab. If it is unique to lactating epithelial cells, an antibody will be prepared and used as cell discriminant. The possibility of using unique isoenzymes to distinguish normal from neoplastic cells will be explored (see Hilf project). The use of immunological specificity for cell identification is the method of choice since the capacity to produce casein and lactose is frequently lost when epithelial cells of mammary origin are cultured.

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 1)

Finding a marker for each cell type within a heterogenous tumor would be helpful to the pathologist as well as the cell biologist and biochemist who must study the products of the whole tumor as produced by the individual cell types.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino

Program: Breast Cancer Exper. Biology Site Visit Date: March 12, 1973

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$81,071 requested

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Cultivation of Normal and Malignant Human Mammary Tissue: Hormone Dependent Susceptibility of Mammary Tissue to Transformation

Principal Investigator: Edwin V. Gaffney, Ph.D.
Name/Address The Pennsylvania State University
Performing 219 Life Sciences I
Organization: University Park, Pennsylvania 16802

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: To develop human mammary cell lines, to transform them with SV40 or benzopyrene, to study the hormonal response in vitro of human neoplastic cells.

Approach: Single cell suspensions are prepared from tissues using hyaluronidase or collagenase or simply by harvesting cells present in the exudates formed on the cut surface of tumor tissue. The collected cells are separated by sedimentation at unit gravity in bovine albumin gradients. Cultivation of individual fractions will be carried on adding 1% methylcellulose in the culture fluid and using as support 7% acrylamide gel prepared as for electrophoresis and "detoxicated." Two rat mammary cell lines have been established in the author's lab. using this procedure which appears to reduce fibroblast overgrowth. If human cell lines can be obtained with the same procedure, they will be monitored for casein and lactose synthetase B protein under the supervision of Dr. S. Patton. The sedimentation at unit gravity will be carried on in collaboration with Dr. Hymer. The pathology of human mammary tissue will be monitored by Dr. Magnani. The human cell lines will be transferred by SV40 and/or benzopyrene following a procedure which permitted the author to transform human amnion cells. The "response" of transformed cells to hormones will be studied in terms of culture growth and DNA synthesis and, as comparison, the response of established lines from human mammary carcinomas will be evaluated. Six of these cell lines are available to the author.

Progress: New Contract

Significance for Cancer Research (NCP Objective 5 Approach 6)

Through in vitro cultivation of human mammary tissue, normal and abnormal, determine characteristics of cancer that produce different growth rates which may be influenced by viruses, carcinogens and/or hormones.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date: March 28, 1973

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$56,506 estimated

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: Studies to Determine the Pathological History of the Mammary Gland in Pseudohermaphroditic Rats and Mice

Principal Investigator: Susumu Ohno, Ph.D.
Name/Address: City of Hope Medical Center
Performing: 1500 East Duarte Road
Organization: Duarte, California 91010

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: To study the consequence of the testicular feminization mutation on the mammary gland and the effect of androgen "insensitivity" on the development of the secretory patterns of pituitary gonadotrophins.

Approach: A colony of Tfm/Y ♂ will be examined for number and conformation of nipples and breast development. Tfm/O ♀ will be mated, colostrogenesis, lactogenesis, and litter growth, will be followed. The behavior of Tfm mammary gland cells in a male environment. Sex reversion of Tfm/t heterozygotes to males will be obtained by introduction of a Sxr mutant gene which gives testes and male phenotypes to X/X and X/O females. Mediation of Tfm regulatory locus on imprinting a female or male secretory pattern on the hypothalamus-pituitary axis will be studied by analyzing a) LH and prolactin production by estrogen treatment of Tfm/Y ♂; b) the influence of testosterone given to new born on LH and prolactin production; c) the level of androgen binding by polysomes in the cytosol or by nuclear fractions, using testosterone, DHT and 5 α -androstane-3 α -17 β -diol; d) the estrogen receptor neurons and androgen binding protein in the hypothalamus at neonatal stage. The role of testosterone in mammary cell death will also be studied in a tumor which is testosterone dependent for growth in vivo but not in vitro. Mutants such as Tfm/Y ♂, Tfm/O ♀, Tfm/+, Sxr/+ ♂, and Tfm/O, Sxr/+ ♂, derived from a Tfm for mammary tumor presence.

Progress: New Contract

Significance for Cancer Research (NCP Objective 1 Approach 4)
Study of an unusual animal model which may contribute valuable information to the etiology of hormonal responsive of mammary cancer.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.
Program: Breast Cancer Exper. Biology Site Visit Date: March 10, 1973
Technical Review Group: Breast Cancer Experimental Biology Committee
Relevance Review Group: Breast Cancer Steering Committee
FY73 Funds: \$88,802 requested

April 26, 1973

CONTRACT RESEARCH SUMMARY

Title: The Response of the Embryonic Mammary Gland to Androgenic Hormones

Principal Investigator: Klaus Kratochwil, Ph.D.

Name/Address Institute for Molecular Biology, Austrian Academy of Sciences,

Performing 5020 Salzburg, Akademiestrasse 26

Organization: Salzburg, Austria

Contract Number:

Starting Date: June 1973

Expiration Date: June 1974

Goal: To study the acquisition of hormone responsiveness during development of the mammary gland.

Approach: In previous work it has been demonstrated that the mammary gland develops along the female-type unless androgens are produced by the fetus. The action of testosterone on the mammary gland will be analyzed to establish whether sensitivity to the hormone exists during the whole developmental period or whether it is acquired at any given time. This generation will be studied by explanting mammary gland buds in several areas of the same embryo or in other embryos at various stages of development. Determination of the developmental stage will be followed by evaluating binding and concentration of testosterone to the mammary tissue. The role of the stroma in determining the androgen-induced atrophy of the mammary gland will be studied by separation and recombination of epithelial and mesenchymal components from different subjects at different stages of development. EM analysis of the epithelio-mesenchymal interface will be done on morphological comparison between the appearance of regressing mammary cells and the effects of collagenase, hyaluronidase, actinomycin D, and cycloheximide on the destruction of mammary rudiments will be carried on as a preliminary study to understand the mechanism of cell death due to testosterone action.

(Interaction of this project with Drs. Ohno and Kan project on the behavior of the mammary gland in mice with testicular feminization mutation is planned)

Progress: New Contract

Significance for Cancer Research (NCP Objective 3 Approach 3)

Should provide very basic information on the development of hormonal responsiveness in normal and neoplastic cells.

Project Officer: D. Jane Taylor, Ph.D. and Pietro M. Gullino, M.D.

Program: Breast Cancer Exper. Biology Site Visit Date:

Technical Review Group: Breast Cancer Experimental Biology Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$22,350 requested

CONTRACT RESEARCH SUMMARY

Title: Experimental Design and Statistical Services for Studies in the Treatment of Primary Breast Cancer

Principal Investigator: C. A. McMahan, Ph.D.

Name/Address Louisiana State University

Performing 1542 Tulane Avenue

Organization: New Orleans, Louisiana

Contract Number: PH43-NCI-65-573

Starting Date: 2/15/65

Expiration Date: 3/31/74

Goal: To provide statistical management, consultation and services for studies of recurrence and survival in primary female breast cancer being carried out by certain members of the Cooperative Breast Cancer Study Group.

Approach: The principal investigator participates in the planning of studies to be carried out and manages the collection, analysis, and evaluation of data pertaining to preliminary studies of accuracy and reproducibility of methods and to major studies which are carried on by the Study Group. He and his staff design forms for data collection, supervise the labeling and handling of specimens for laboratory analysis on a blind basis, and collect and analyze the resulting data. Laboratory and clinical data are reduced to punch cards or computer tape for tabulation and analysis. Reports are prepared for the guidance of the investigators.

Progress: This contractor has demonstrated capability in the operation of a statistical center for this collaborative study of relating clinical, pathologic and urinary steroid factors to risk of recurrence in breast cancer. He has successfully standardized procedures for both the urinary steroid laboratory and the pathology laboratory so that reproducible results are obtained. Preliminary analyses of the data on hand have been done and there is indication that several of the factors being evaluated are related to risk of recurrence.

Significance for Cancer Research (NCP Objective 6 Approach 6)

Project Officer: Max H. Myers, Ph.D.

Program: Breast Cancer Diagnosis

Site Visit Date: Oct. 17, 1972

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$89,556

CONTRACT RESEARCH SUMMARY

Title: Automated Methods for Determination of Steroid Metabolites

Principal Investigator: Dr. Federico Welsch
Name/Address The Worcester Foundation for Experimental Biology
Performing 222 Maple Avenue
Organization: Shrewsbury, Mass. 01545

Contract Number: 66-968

Starting Date: 6/10/73

Expiration Date: 6/9/74

Goal: To develop automated analysis for determination of urinary steroid hormones.

Approach: This analysis of steroid hormones is based on paper chromatography with uniquely designed triple wavelength readout permitting an automated Allen correction to be incorporated. The methodology is highly specific and reproducible, permitting the analysis of a spectrum of hormonal metabolites on 5-10% aliquot of a 24-hour specimen.

Progress: In the past year the contractor exceeded the specifications for work load by 200% without additional cost. The laboratory serves as the analytical arm of the clinical study designed to develop a predictive index for spotting early recurrences after radical mastectomy.

Significance for Cancer Research (NCP Objective 6 Approach 6)

This contract represents one of our two service components to the clinical studies.

Project Officer: Ihor J. Masnyk, Ph.D.

Program: Breast Cancer Diagnosis

Site Visit Date: 1971

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$220,000

CONTRACT RESEARCH SUMMARY

Title: Steroid Excretion Study in Patients With Breast Cancer

Principal Investigator: Dr. Per Vestergaard
Name/Address Research Foundation for Mental Hygiene
Performing Rockland State Hospital
Organization: Orangeburg, N.Y. 10962

Contract Number: 67-1465

Starting Date: 6/28/73

Expiration Date: 6/27/74

Goal: To develop automated analysis for determination of urinary steroid hormones.

Approach: The analysis of steroid hormones is based on column chromatography, the column consisting of fine, capillary teflon tubings kept in a constant temperature bath. After the urine extracts are deposited at the head of the column, automation takes over so that all steps become highly reproducible. Even though the analysis per se is nonspecific, the high number of fractions collected allows for fine separation of metabolites without cross-contamination.

Progress: In the last year the contractor exceeded the expected work load by 10%. Several studies are based on the data from this laboratory: Adrenalectomy Prediction Study, High Risk Study Based on Israel Ethnic Groups, Cyclic Excretion of Steroid Hormones, Endometrial Cancer Study.

Significance for Cancer Research (NCP Objective 6 Approach 6)

This contract represents one of our two efforts in the area of steroid analysis, serving several clinical studies.

Project Officer: Thor J. Masnyk, Ph.D.

Program: Breast Cancer Diagnosis

Site Visit Date: March 28, 1972

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$84,000

CONTRACT RESEARCH SUMMARY

Title: Urinary Steroid Hormone Excretion Patterns in Ethnic Groups With Widely Different Breast Cancer Incidence

Principal Investigator: Dr. Jack Gross

Name/Address Hebrew University

Performing Hadassah Medical School

Organization: P.O. Box 1172

Jerusalem, Israel

Contract Number: 71-2016

Starting Date: 10/26/72

Expiration Date: 10/25/73

Goal: To determine steroid hormone excretion patterns in three Israel ethnic groups with divergent cancer incidence rate.

Approach: Postmenopausal women from East European origin, from Yemen and North Africa are studied. Twenty-four hour urine specimens are collected and analyzed for the content of androgens and corticoid metabolites. The results are related to cancer incidence rate in these three groups (from 10 in case of Yemen women to 65 for East Europeans).

Progress: About 150 women have been entered on study, the goal being 75 women per ethnic group. Analytical data obtained was correlated with the country of birth of the subjects; several items were significantly correlated even at this preliminary attempt at analysis.

Significance for Cancer Research (NCP Objective 5A Approach 1)
Attempt to better characterize high risk groups.

Project Officer: Ihor J. Masnyk, Ph.D.

Program: Breast Cancer Diagnosis

Site Visit Date: October, 1971

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$31,500

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Patient Input-Follow-up

Principal Investigator: Dr. Robert W. Talley

Name/Address Henry Ford Hospital

Performing 2799 West Grand Blvd.

Organization: Detroit, Michigan 48202

Contract Number: 72-3230

Starting Date: 4/3/73

Expiration Date: 4/2/74

Goal: To follow up clinical cases entered in the Primary Breast Cancer Study.

Approach: Primary Breast Cancer Study is aimed at the development of a predictive index for early recurrences after radical mastectomy. One of the more important aspects of the study is the input of eligible patients who are to be followed up for 2 years in regard to possible recurrence of the disease.

Progress: About 30 patients were to be entered by the contractor in the first contract year. This year's contract is devoted to follow-up. The patients are being entered.

Significance for Cancer Research (NCP Objective 6 Approach 6)

Having a prognostic handle on the course of the disease after mastectomy would help in planning further adjuvant/prophylactic therapy.

Project Officer: Ihor J. Masnyk, Ph.D.

Program: Breast Cancer Diagnosis

Site Visit Date: March 2, 1972

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$9,720

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Study Patient Input (Follow-up)

Principal Investigator: Dr. Anne C. Carter
Name/Address Research Foundation of the State of New York
Performing 450 Clarkson Avenue
Organization: Brooklyn, N.Y. 11203

Contract Number: 72-3231

Starting Date: 4/16/73

Expiration Date: 4/15/74

Goal: To follow up clinical cases entered in the Primary Breast Cancer Study.

Approach: Primary Breast Cancer Study is aimed at the development of a predictive index for early recurrences after radical mastectomy. One of the more important aspects of the study is the input of eligible patients who are to be followed up for 2 years in regard to possible recurrence of the disease.

Progress: About 70 patients were to be entered by the contractor in the first contract year. This year's contract is devoted to follow-up. The patients are being entered.

Significance for Cancer Research (NCP Objective 6 Approach 6)
Having a prognostic handle on the course of the disease after mastectomy would help in planning further adjuvant/prophylactic therapy.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: June 8, 1972
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY73 Funds: \$50,000

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Study Patient Input (Follow-up)

Principal Investigator: Dr. Carleton R. Haines
Name/Address University of Vermont College of Medicine
Performing Medical Center Hospital of Vermont
Organization: Burlington, Vermont 05401

Contract Number: 72-3232

Starting Date: 3/13/73

Expiration Date: 3/12/74

Goal: To follow up clinical cases entered in the Primary Breast Cancer Study.

Approach: Primary Breast Cancer Study is aimed at the development of a predictive index for early recurrences after radical mastectomy. One of the more important aspects of the study is the input of eligible patients who are to be followed up for 2 years in regard to possible recurrence of the disease.

Progress: About 25 patients were to be entered by the contractor in the first contract year. This year's contract is devoted to follow-up. The patients are being entered.

Significance for Cancer Research (NCP Objective 6 Approach 6)

Having a prognostic handle on the course of the disease after mastectomy would help in planning further adjuvant/prophylactic therapy.

Project Officer: Thor J. Masnyk, Ph.D.

Program: Breast Cancer Diagnosis

Site Visit Date: June 9, 1972

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$8,000

CONTRACT RESEARCH SUMMARY

Title: Steroid Hormone Excretion Patterns (Cyclic and Daily Variations)

Principal Investigator: Dr. Anne C. Carter
Name/Address Research Foundation of the State of New York
Performing 450 Clarkson Avenue
Organization: Brooklyn, N.Y. 11203

Contract Number: 72-3851

Starting Date: 4/16/73

Expiration Date: 4/15/74

Goal: To determine fluctuations of daily excretion of urinary steroid hormones during one cycle.

Approach: Several studies are under way searching for prognostic indexes based on urinary steroid hormone excretions. One, two or four day urines are collected to give a representative value for a patient. No solid background data are available on daily fluctuations of hormonal excretion. This contract should produce this knowledge.

Progress: Last year 10 postmenopausal women collected urines for 30 days. Daily collections were sent for analysis of androgens, corticoids and estrogens.

Significance for Cancer Research (NCP Objective 6 Approach 6)
Background data case for several studies.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: June 8, 1972
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY73 Funds: \$20,000

CONTRACT RESEARCH SUMMARY

Title: Breast cancer tissue supply

Principal Investigator: Dr. Paul O'Brien
Name/Address Medical College of South Carolina
Performing Charleston, South Carolina
Organization:

Contract Number: NIH-NCI-G-72-3867
Starting Date: 6/30/72 Expiration Date: 6/30/74
Goal: To obtain tissue and blood specimens from patients with breast cancer, for use in immunodiagnostic assays.

Approach: At the time of surgery, obtain tumor and normal breast tissue. Obtain serum and peripheral blood lymphocytes before surgical therapy and at regular intervals thereafter. These specimens are shipped promptly to other investigators in the program, for use in immunodiagnostic assays.

Progress: During the first contract year, the contractor has supplied more than the minimum number of specimens specified. The logistics for obtaining specimens and for satisfactory shipment have been worked out successfully.

Significance for Cancer Research (NCP Objective 5B Approach 4)
Specimens from breast cancer patients are needed for rapid development of potential immunodiagnostic assays.

Project Officer: Dr. Ronald B. Herberman
Program: Breast Cancer Diagnosis Site Visit Date: May 1972
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY73 Funds: \$60,000

CONTRACT RESEARCH SUMMARY

Title: Tissue Culture Lines from Patients with Breast Cancer

Principal Investigator: Dr. Etienne Lasfargues
Name/Address: Institute for Medical Research
Performing: Camden, New Jersey
Organization:

Contract Number: NIH-NCI-G-72-3868

Starting Date: June 1, 1972

Expiration Date: June 1, 1974

Goal: To develop techniques for more reliably growing tumor cells and normal control cells from breast cancer patients in tissue culture, and to supply these cells for use in immunodiagnostic assays.

Approach: Tumor cells and normal breast tissue cells are obtained and placed into tissue culture. A variety of procedures for harvesting the cells will be attempted and various media and supplements will be tested for their ability to support cell viability and growth. Primary culture cells and established cell lines will be frozen in viable condition and either fresh or frozen cells will be made available to other investigators in the program for use in immunodiagnostic assays.

Progress: During the first contract year, the logistics for obtaining tissue specimens from local sources and from other NCI contractors have been well worked out. About 50% of the tumor cells received have been successfully maintained in culture for short periods of time. Some cells have now persisted in culture for over six months. Only limited proliferation of epithelial cells has been seen thus far. After initial difficulties in freezing primary culture cells, the contractor can successfully do this in most instances. Cells have been provided to other investigators for use in cell-mediated cytotoxicity assays.

Significance for Cancer Research (NCP Objective 5B Approach 4)

Tissue cultures of tumor cells are essential for development of cell-mediated cytotoxicity assays, which have potential usefulness in the immunodiagnosis of cancer.

Project Officer: Dr. Chou-Chik Ting

Program: Breast Cancer Diagnosis

Site Visit Date: May 1972

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$65,000

CONTRACT RESEARCH SUMMARY

Title: Tissue culture lines from patients with breast cancer

Principal Investigator: William Reeves and Relda Cailleau
Name/Address M. D. Anderson Hospital and Tumor Institute
Performing Houston, Texas 77025
Organization:

Contract Number: NIH-NCI-G-72-3869

Starting Date: 6/1/72

Expiration Date: 6/1/74

Goal: To develop techniques for more reliably growing tumor cells and normal control cells from breast cancer patients in tissue culture, and to supply these cells for use in immunodiagnostic assays.

Approach: Tumor cells and normal breast tissue cells are obtained and placed into tissue culture. A variety of procedures for harvesting the cells will be attempted and various media and supplements will be tested for their viability and growth. Primary culture cells and established cell lines will be frozen in viable condition and either fresh or frozen cells will be made available to other investigators in the program for use in immunodiagnostic assays.

Progress: Complete tissue culture facilities have been set up and the logistics for obtaining tissue specimens and for shipping specimens and culture cells have been worked out. Cells from both primary breast tumors and from malignant pleural effusions have been placed into culture. In a collaborative project, aliquots of the same materials have been sent to Dr. Lasfargues and to Dr. Grace Cannon. This has allowed comparison in culture techniques and in interpretation of cell outgrowths. Most of the biopsy cells and the cells in culture have been examined carefully by electron microscopy.

Significance for Cancer Research (NCP Objective 5B Approach 4)
Tissue cultures of tumor cells are essential for development of cell-mediated cytotoxicity assays, which have potential usefulness in the immunodiagnosis of cancer.

Project Officer: Dr. Ronald B. Herberman

Program: Breast Cancer Diagnosis

Site Visit Date: May 1972

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$124,900

CONTRACT RESEARCH SUMMARY

Title: Development of Immunological Assays for Human Breast Cancer

Principal Investigator: Dr. Anthony Monaco
Name/Address: Department of Surgery
Performing: Boston City Hospital
Organization: Harvard Medical School
Boston, Massachusetts

Contract Number: NIH-NCI-G-72-3870

Starting Date: June 20, 1972

Expiration Date: June 20, 1974

Goal: To perform a series of assays of cell-mediated immunity in patients with breast cancer and to determine their usefulness in prognosis and in detecting residual disease.

Approach: Patients with breast cancer are being tested for reactivity in several assays of cell-mediated immunity: skin reactivity to a standard battery of recall antigens and to extracts of breast tumor cells, sensitization to dinitrochlorobenzene, cell-mediated cytotoxicity against tissue culture cells derived from breast tumors, lymphocyte stimulation by tumor cell extracts, and inhibition of leukocyte migration by tumor cell extracts. These results are being correlated with the clinical stage of disease and response to therapy.

Progress: All the above assays have now been set up and data is being accumulated. Many of the technical problems associated with these assays have been worked out.

Significance for Cancer Research (NCP Objective 5B Approach 4)

Assays for cell-mediated immunity can be potentially useful for diagnosis and prognosis.

Project Officer: Ronald B. Herberman, M.D.

Program: Breast Cancer Diagnosis

Site Visit Date:

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$80,000

CONTRACT RESEARCH SUMMARY

Title: Purification of an Antigen from Human Mammary Carcinoma

Principal Investigator: Dr. Donald Marcus

Name/Address Associate Professor of Medicine,

Performing Microbiology and Immunology

Organization: Albert Einstein College of Medicine

Bronx, New York 10461

Contract Number: NIH-NCI-G-72-3871

Starting Date: June 15, 1972

Expiration Date: June 14, 1974

Goal: To identify antigens associated with human breast cancer and to develop a quantitative assay for this antigen, for potential use in the immunodiagnosis of breast cancer.

Approach: The contractor's approach is to develop antisera specific for the antigen(s) and to purify the antigen(s). Tests for specificity will be performed with breast tumor tissues, normal breast tissues, and other control tissues. Contaminating antibodies or antigens will be removed by immunoadsorbents and by fractionation procedures. Isolated and purified antigen will be labelled with ^{125}I and used in a double antibody radio-immunoassay.

Progress: During the first contract year, the contractor has produced an antiserum which reacts with extracts of primary and metastatic breast cancer and does not react with similar extracts of normal breast tissue. A single band is seen on immunodiffusion. This antiserum has been used in affinity column chromatography for isolation of the antigen. Further purification of the antigen has been accomplished by iso-electric focusing in acrylamide gel.

Significance for Cancer Research (NCP Objective 5B Approach 4)

Immunodiagnostic assays offer the potential for early diagnosis of breast cancer.

Project Officer: K. Robert McIntire, M.D.

Program: Breast Cancer Diagnosis

Site Visit Date:

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$45,000

CONTRACT RESEARCH SUMMARY

Title: Ultrasound Mammography

Principal Investigator: Dr. Gilbert Baum
Name/Address Albert Einstein College of Medicine
Performing 1300 Morris Park Avenue
Organization: Bronx, N.Y. 10461

Contract Number: 72-3872

Starting Date: 6/20/73

Expiration Date: 6/19/74

Goal: To develop ultrasonic diagnostic criteria for malignant breast lesions.

Approach: Ultrasonic "viewing" has had highly successful application for diagnostic purposes in some organs. The contractor is world's authority on the use of ultrasonics in eye examinations. The need for nonradiational detectors of breast tumors is very high. The contractor's aim was to develop a new scanner suitable for breast work, and to lay foundation for ultrasonic mammography surveys.

Progress: Preliminary designs of the new scanner have been experimented with. Actual construction was hampered by lack of suitable commercially produced units, making the timetable some 3-6 months behind schedule. Breast examinations were carried out on prototype eye instruments.

Significance for Cancer Research (NCP Objective 5 Approach 4)

A new modality for diagnostic purposes.

Project Officer: Ihor J. Masnyk, Ph.D.

Program: Breast Cancer Diagnosis

Site Visit Date: 3/21/73

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$102,478

CONTRACT RESEARCH SUMMARY

Title: Isotope Source for Mammography

Principal Investigator: Dr. Harold L. Atkins
Name/Address Brookhaven National Laboratory
Performing Upton, New York 11973
Organization:

Contract Number: 73-301

Starting Date: 11/20/72

Expiration Date: 10/31/73

Goal: Production of a new source for mammography based on technetium-97m.

Approach: A new source for the radiation energy to be used in mammography is to be produced, basing it on technetium-97m. This source produces discrete photon energies in the desired energy range so that image with increased contrast could be obtained at a lower radiation dose. The characteristics of this source are to be determined in terms of film contrast and resolution. The quality of examination is to be compared to conventional X-ray examination.

Progress: Progress has been hampered by a temporary shutdown of the plant.

Significance for Cancer Research (NCP Objective 5 Approach 4)
A new source for mammography.

Project Officer: Ihor J. Masnyk, Ph.D.

Program: Breast Cancer Diagnosis

Site Visit Date: -

Technical Review Group: Breast Cancer Diagnosis Committee

Relevance Review Group: Breast Cancer Steering Committee

FY73 Funds: \$69,205

CONTRACT RESEARCH SUMMARY

Title: Analysis of Breast Secretions

Principal Investigator: Dr. N. L. Petrakis
Name/Address University of California
Performing 3rd and Parnassus
Organization: San Francisco, California 94122

Contract Number: Contract is being negotiated
Starting Date: N/A Expiration Date: N/A
Goal: To evaluate Sartorius breast pump technique as a means of breast cancer detection, using cytological biochemical and virological analysis of secreted material.

Approach: Obtain breast secretions by means of a pump developed by Dr. O. Sartorius. Secretions will be analyzed by cytological, virological and biochemical methods, and correlated to mammographic and pathological findings. In select cases breast intraductal catheterization will be carried out to pinpoint locations of suspected lesions.

Progress: The contract is being negotiated.

Significance for Cancer Research (NCP Objective 5 Approach 4)
Extend the cytologic and biochemical detection methods.

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: Oct. 19, 1972
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY73 Funds: \$179,841

CONTRACT RESEARCH SUMMARY

Title: Electronic Mammography

Principal Investigator: Dr. D. Sashin
Name/Address University of Pittsburgh School of Medicine
Performing Pittsburgh, Pa. 15213
Organization:

Contract Number: Contract is being negotiated.
Starting Date: N/A Expiration Date: N/A
Goal: Develop electronic radiography technique designed for mass screening for early breast cancer detection.

Approach: Electronic radiography involves substitution of image intensification and TV techniques for X-ray film, increasing the efficiency with which X-rays can be recorded, thus reducing the X-ray dose. The program consists of two parts: (1) design and construction of a prototype system optimized for visualization of soft tissues and (2) evaluation of the system regarding its ability to display clinical details relative to present mammography systems.

Progress: Contract will start in late spring '73.

Significance for Cancer Research (NCP Objective 5 Approach 3)

Project Officer: Ihor J. Masnyk, Ph.D.
Program: Breast Cancer Diagnosis Site Visit Date: Jan. '73
Technical Review Group: Breast Cancer Diagnosis Committee
Relevance Review Group: Breast Cancer Steering Committee
FY73 Funds: \$124,349 (subject to negotiation)

CONTRACT RESEARCH SUMMARY

Title: Endocrine Tumor Screening

Principal Investigator: Dr. Arthur E. Bogden
Name/Address: Mason Research Institute
Performing: 20 Harvard Street
Organization: Worcester, Massachusetts 01608

Contract Number: PH43-NCI-G-65-6

Starting Date: 11/1/65

Expiration Date: 7/31/73

Goal: To carry out preclinical evaluations of single therapeutic modalities and combinations of modalities, e.g., surgery, chemotherapy, and immunotherapy, in experimental mammary tumors of known biological characteristics resembling those of human breast cancer.

Approach: The studies are being carried out in four transplantable rat mammary tumors. Two of the mammary tumors will be utilized for evaluating: 1) some compounds known to effect serum prolactin levels of the hosts, e.g., ergot derivatives, L-Dopa, etc.; 2) 10 to 12 agents known to be of clinical effectiveness in some breast cancers as suggested by the Chairman, Breast Cancer Treatment Committee, e.g., Cytosan, Methotrexate, 5-Fluorouracil, Thiotepe, etc.; 3) antiviral agents. The compounds will be tested for activity in inhibiting early phases of tumor growth, as well as for inducing regression of the 13762 tumor which metastasizes routinely to the lungs and other organs of the host and their effects on host hormone levels, e.g., prolactin, FSH, LH, etc. A small amount of work continues on immunotherapy with the methanol-soluble fraction of Mycobacterium butyricum (MSF-MB) as a non-specific immune stimulating agent.

Progress: From the time of initiation of this contract (1965) to July 1972 the contract has had two major objectives: a. Evaluation of steroidal and non-steroidal agents for endocrine potencies; b. Evaluation of the same agents for tumor inhibitor effects in endocrine-related tumor systems. During this period of time over 50 papers have been published on the results obtained under the contract, and data obtained from the hormonal assay program have been published in three monographs. Results from the tumor program have been presented at the last five annual meetings of the American Association of Cancer Research. Recent site visitors were interested in the possible use of the 13762 tumor as a surgical adjuvant model; they were also impressed by the MSF-MB immunotherapy experiments.

Significance for Cancer Research (NCP Objective 6 Approach 6.1)

Evaluate methodologies of immunology, chemotherapy, surgery, and irradiation (alone and in combinations) in experimental mammary tumor systems which could contribute to information for the treatment of human breast cancer.

Project Officer: Dr. D. Jane Taylor

Program: Breast Cancer Treatment

Site Visit Date: 3/22/73

Technical Review Group: Breast Cancer Treatment Committee

Relevance Review Group: Breast Cancer Task Force Steering Committee

FY73 Funds: \$194,200

CONTRACT RESEARCH SUMMARY

Title: Develop a Method for Predicting Response to Adrenalectomy

Principal Investigator: Dr. Elwood Jensen

Name/Address University of Chicago

Performing 5801 Ellis Avenue

Organization: Chicago, Illinois 60637

Contract Number: PH43-NCI-G-66-945

Starting Date: 6/16/72

Expiration Date: 6/15/73

Goal: To develop a test for endocrine-dependent cancer by determining the level of estrogen receptor (estrophilic protein) in biopsy material.

Approach: Breast cancer has long been considered either "hormone-dependent" or autonomous, but any clinical test for such a distinction has been lacking. Since 1967 Dr. Jensen has been studying estrogen receptors and more recently has been attempting to correlate estrophile level with response to endocrine therapy. As of several months ago the possibility of such a correlation appeared good. Fifty-one patients with metastatic disease received treatment in form of ablative surgery or hormonal drugs. Of 32 patients with a negative receptor pattern only one responded to therapy; of 5 with "borderline" patterns only one responded; but 11 out of 14 with a positive pattern responded positively to therapy.

Progress: Since the last annual report Dr. Jensen has examined 177 specimens for the presence of receptors, more than in the five previous years cumulatively because of improved methods. Of these 78 were primary tumors, 35 benign tumors, and 16 still under examination. The correlation of clinical response to receptor level has continued to hold up: of 32 patients without receptor only one had a remission, while 16 of 20 with receptor-containing tumors had remissions after treatment.

Significance for Cancer Research (NCP Objective 5(B) Approach 6)

Project Officer: Dr. Erwin P. Vollmer

Program: Breast Cancer Treatment

Site Visit Date: 10/8/68

Technical Review Group: Breast Cancer Treatment Committee

Relevance Review Group: Breast Cancer Task Force Steering Committee

FY73 Funds: \$78,056

P.I. reported in seminars 10/10/68, 2/27/69 & 2/23/71

CONTRACT RESEARCH SUMMARY

Title: Primary Breast Cancer Group (NSABP)

Principal Investigator: Bernard Fisher, M.D.

Name/Address University of Pittsburgh

Performing School of Medicine, Department of Surgery

Organization: 3550 Terrace Street

Pittsburgh, Pennsylvania 15213

Contract Number: NIH-NCI-G-72-3876

Starting Date: 6/30/72

Expiration Date: 6/29/74

Goal: To determine the relative merits of total (simple) mastectomy and radical mastectomy, and whether prolonged chemotherapy after mastectomy is beneficial in primary breast cancer.

Approach: Patients with operable cancer of the breast are designated as clinically negative or positive nodes and then randomly assigned (A) with negative nodes: (1) radical mastectomy, (2) total mastectomy, or (3) total mastectomy with regional radiation; (B) with positive nodes: (1) radical mastectomy or (2) total mastectomy with regional radiation. In the chemotherapy study patients with four or more metastatic axillary nodes will be given radical mastectomy followed by (1) L-Phenylalanine Mustard 0.15 mg/kg/day, p.o., x 5 q. 6 weeks; or (2) placebo in equivalent dosage. Chemotherapy begins 2-4 weeks after mastectomy and continues until treatment failure is recognized.

Progress: Thirty-two institutions are now participating in the mastectomy study and 19 additional institutions in the chemotherapy study. As of November 10, 1972, 669 patients had been entered in the mastectomy study, and the original objective of 1000 will be reached in the current year. However, a new option of simpler surgery is being considered. The chemotherapy study did not start until October 1972, and input has been disappointing, largely because only 25% of patients with primary operable breast cancers having radical mastectomy are eligible. Changes in protocol that would increase entry are being considered.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Dr. Paul Carbone, Dr. Alfred Ketcham

Program: Breast Cancer Treatment

Site Visit Date: 1/17/72

Technical Review Group: Breast Cancer Treatment Committee

Relevance Review Group: Breast Cancer Task Force Steering Committee

FY73 Funds: \$499,833

CONTRACT RESEARCH SUMMARY

Title: An Evaluation of Surgical Adjuvant Chemotherapy Utilizing 5-Fluorouracil, Cytosan and Prednisone in Patients with Breast Cancer.

Principal Investigator: Dr. David L. Ahmann
Name/Address Mayo Clinic
Performing Rochester, Minnesota 55901
Organization:

Contract Number:

Starting Date: 7/1/73

Expiration Date: 6/30/74

Goal: To assess the effects of surgical adjuvant chemotherapy, utilizing a multiple drug program in patients with locally advanced mammary carcinoma.

Approach: Patients with unfavorable primary breast cancers will be randomly assigned to treatment programs involving surgery alone, surgery followed by chemotherapy, surgery followed by postoperative radiation therapy and adjuvant chemotherapy. High risk patients with four or more positive nodes will have surgery including complete removal of the breast and axillary contents with preservation of the pectoral muscles if possible. After suitable stratification (according to menstrual category, tumor size, nodal involvement) patients will be assigned to radiation and chemotherapy treatment groups. Adjuvant chemotherapy will be provided two weeks postoperatively while the patient is undergoing radiation (when this is given): 5-Fluorouracil and Cyclophosphamide by rapid intravenous infusion for 5 days and Prednisone orally for 5 days. The treatments will be repeated every six weeks until evidence of recurrent or metastatic disease occurs.

Progress: Project on this protocol has not yet started. Preliminary studies suggest a good response rate (about 50%) for this therapeutic combination.

Significance for Cancer Research (NCP Objective 6 Approach 1)

Project Officer: Dr. Paul Carbone

Program: Breast Cancer Treatment

Site Visit Date: To be set up

Technical Review Group: Breast Cancer Treatment Committee

Relevance Review Group: Breast Cancer Task Force Steering Committee

FY73 Funds: \$93,680

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator: Dr. Joseph Post

Name/Address New York University Research Service

Performing Goldwater Memorial Hospital

Organization: Welfare Island, N.Y.

New York, N. Y. 10017

Contract Number:

Starting Date: 7/1/73

Expiration Date: 6/30/74

Goal: To provide information concerning cell cycle parameters in patients with breast cancer.

Approach: In vivo labeling with tritiated thymidine will be coupled with in vitro labeling with C^{14} -thymidine. Where feasible, multiple analyses will be performed on an individual patient. Standard radiographic techniques will be utilized to accumulate the data. New mathematical models are being designed by Dr. Post and Dr. Simon; these will be utilized for analysis of the data.

Progress:

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Dr. Douglass Tormey

Program: Breast Cancer Treatment

Site Visit Date: 3/9/73

Technical Review Group: Breast Cancer Treatment Committee

Relevance Review Group: Breast Cancer Task Force Steering Committee

FY73 Funds: \$108,000

CONTRACT RESEARCH SUMMARY

Title: Cell Kinetics of Breast Cancer

Principal Investigator: Dr. H. H. Sky-Peck

Name/Address Rush-Presbyterian-St. Luke's Medical Center

Performing Department of Biochemistry

Organization: 1753 West Congress Parkway

Chicago, Illinois 60612

Contract Number:

Starting Date: 7/1/73

Expiration Date: 6/30/74

Goal: The goal of this contract is to provide estimates of cell cycle time parameters, and to relate these parameters to therapeutic results.

Approach: The approach which will be used will be that of utilizing tritiated thymidine in vivo pulses with C^{14} -thymidine in vitro pulses followed by autoradiographic analysis. Clinical coordination will be done retrospectively by comparing the cell kinetic data with the therapeutic modalities employed and the results of treatment.

Progress:

Significance for Cancer Research (NCP Objective 5 Approach 6)

Project Officer: Dr. Douglass Tormey

Program: Breast Cancer Treatment

Site Visit Date: 5/9/73

Technical Review Group: Breast Cancer Treatment Committee

Relevance Review Group: Breast Cancer Task Force Steering Committee

FY73 Funds: \$43,855

CONTRACT RESEARCH SUMMARY

Title: Prolactin Radioimmunoassays in Human Serum

Principal Investigator: Dr. W. P. VanderLaan
Name/Address Scripps Clinic and Research Foundation
Performing 476 Prospect Street
Organization: La Jolla, California 92037

Contract Number:

Starting Date: Expiration Date:

Goal: To provide 1000 radioimmunoassays of prolactin in human serum per month in collaborative research on breast cancer.

Approach: Human prolactin (h PRL) has been used to produce antibodies in rabbits by techniques of Goudie et al. and for radioiodination (^{125}I) construction of standard curves. Antibody on hand is suitable for assay purposes in initial dilutions of 1:20,000 and 1:40,000. Sera will be assayed in duplicate and assays will be run at two to four weekly intervals according to volume of work. Protocols for research will be agreed upon by the Principal Investigator, cooperating clinicians; the Project Officer and a representative of the Treatment Committee.

Progress: The methodology is ready. The project is about to begin as soon as funds are available.

Significance for Cancer Research (NCP Objective 5(A) Approach 1)

Project Officer: Dr. Erwin P. Vollmer
Program: Breast Cancer Treatment Site Visit Date:
Technical Review Group: Breast Cancer Treatment Committee
Relevance Review Group: Breast Cancer Task Force Steering Committee
FY73 Funds: \$98,260 (requested)

CANCER DIAGNOSIS CONTRACT PROGRAM

SUMMARY REPORT

July 1, 1972 to June 30, 1973

This recently organized branch has as its goals the detection of small or "early" cancers before they have had a chance to spread to the local or regional lymph nodes or elsewhere. It has been recognized for sometime that cancer of the lung can be diagnosed by cytological means and when a chest x-ray is negative. Until the development of the fiber optic bronchoscopes, it frequently was not possible to detect a small bronchial tumor that was exfoliating cancer cells. Johns Hopkins Medical College physicians have developed and proven that they can localize such tumors and excise them by means of a lobectomy or less. This work was supported by a contract from the Diagnosis Branch. In addition, these physicians have used tantalum powder as a contrast media which is inhaled or blown into the lungs and can demonstrate tumors that are beyond the reach of the bronchoscope. These techniques have now been studied and learned at the Mayo Clinic and are now being studied at the Memorial Hospital in New York City so that these methods can be applied to fairly large populations of high risk, male patients (heavy smokers). These three studies will have sufficient screenees (with half as controls) to demonstrate in about five years that the mortality of lung cancer can or cannot be reduced by 50 per cent by the application of these methods. At the Mayo Clinic hematoporphyrin is being used as a means of tumor localization since if given prior to bronchoscopy a light of a given wave length will produce fluorescence in the area of carcinomatous change.

Patients with colon cancer and other diseases have been studied by means of blood assays for carcinoembryonic antigens (CEA). This has proven of little value as an isolated examination for the diagnosis of colon cancer since it is elevated in a number of benign and non-tumorous diseases. Perhaps sequential examination or examinations of the CEA in the lumen of the bowel may be of more value and such studies are underway. Studies of feces for occult human blood are planned since such examination might be of more value than present studies which must be preceded by a meat free, high roughage diet. Further studies of alpha and gamma fetoproteins in conjunction with blood and bowel lumen levels of CEA may improve the value of screening for small and asymptomatic bowel cancers. Studies of the colon by means of the fiber optic colonoscope as a diagnostic tool in patients with a suspicion of cancer and in whom x-ray studies are negative are

underway. New fiber optic scopes that can be passed to the cecum more rapidly than at present possible are being contemplated.

The branch is considering a number of proposals for diagnosis in the area of thermography, ultrasound, nuclear magnetic resonance, electron spin resonance, vapor and gas sensors and other more exotic proposals that are as yet not well established as diagnostic tools but offer certain possibilities.

Several radiologists at the request of the Diagnosis Branch are studying in several subgroups the present and future x-ray diagnostic modalities available. Groups are engaged in evaluating 1) observer errors; 2) imaging devices; 3) new equipment and machines. Other groups are studying radiological methods for the diagnosis of brain tumors, breast cancer, colon cancer, and lung cancer.

Most of the professional work related to the American Cancer Society-National Cancer Institute Breast Cancer Demonstration Projects has been done by the staff of the Diagnosis Branch, although these projects are funded by the Cancer Control Division. There are now three projects funded, eight approved and nine more to be selected from about 35 applicants in the summer of 1973.

CONTRACT RESEARCH SUMMARY

Title: "Lung Cancer - Early Detection, Localization and Therapy

Principal Investigator: John K. Frost, M.D.

Name/Address Johns Hopkins Hospital

Performing Department of Pathology

Organization: Baltimore, Maryland

Contract Number: NIH-NCI-69-2172

Starting Date: June 28, 1969

Expiration Date: June 27, 1974

Goal: 1) To detect lung cancer by sputum examination and other methods and localize it before it can be seen on conventional x-ray studies.

2) To determine the effect of treatment of small lung tumors on long term survival of patients with lung cancer.

Approach: Persons at high risk for lung cancer such as heavy smokers, certain chemical workers, asbestos workers, and others are being studied by means of sputum cytology and x-ray examination of the chest for the early detection of lung cancer. If cancer is suspected then careful studies are done for tumor localization by means of Tantalum powder, fiber optic bronchoscopy and other localization techniques and treatment by means of wedge resection of a lung or lobectomy is done to "cure" patients with small lung cancers.

Progress: More than 1500 individuals have had over 2300 screenings with a lung cancer rate of 3.9/1000 man years. Improved methods of sputum handling have been developed. Twenty-six lung tumors have been studied for cytogenetic changes and suitable material for study has been obtained in seven. 150 fiber optic bronchoscopies have been performed in the last year and this includes 42 patients with lung cancer. A total of 275 bronchoscopies have been performed since the project started. Most cases of cancer (75%) involved the segmental or more distant bronchi. Teaching files have been developed. Lung cancer can be found when the x-ray examination is negative and resection of a lobe made feasible. It is not known yet if this will prolong life.

Significance for Cancer Research (NCP Objective X Approach)

Recognition of lung cancer before metastasis has occurred and hopefully "cured."

Project Officer: Kenneth B. Olson, M.D.

Program: Lung Cancer Diagnosis

Site Visit Date:

Technical Review Group: Diagnostic Research Advisory Group

Relevance Review Group: Institute Director and Executive Committee

FY73 Funds:

CONTRACT RESEARCH SUMMARY

Title: "Detection and Localization of Bronchogenic Cancer"

Principal Investigator: Robert S. Fontana, M.D.

Name/Address Mayo Clinic

Performing Rochester, Minnesota

Organization:

Contract Number: NIH-NCI-G-72-2000

Starting Date: August 11, 1971

Expiration Date: August 10, 1974

Goal: 1) Improve methods for the early diagnosis of cancer of the lung.
2) Determine the exact improvement in long time survival of patients with lung cancer due to the application of new techniques for early diagnosis.
Approach: A population of high risk patients (i.e., heavy smokers) who are coming to the Mayo Clinic for symptoms other than lung cancer will be examined thoroughly and then put into one of two groups. The studied group will receive an annual chest x-ray examination and sputum examination three times a year that are mailed in. The control group will have an initial chest x-ray examination and a sputum examination and then it will be suggested that they have an annual chest x-ray done near their home and mailed to the Mayo Clinic. It is hoped (but not provided for) that all patients suspected of lung cancer by cytology or x-ray examination will return to the Mayo Clinic for treatment and possible resection of a small cancer.

Screenings with positive sputum for cancer, but with negative chest x-rays will be admitted for exhaustive studies by means of fiber optic bronchoscopy so that localization of a cancer can be done and followed by a wedge resection or less than a pneumonectomy. Computer analysis of chest x-rays is being studied. Hematoporphyrin for localization of tumors is also being studied.

Progress: To date, April 1, 1973 about 2300 persons have been screened and in excess of 500 have returned for a follow-up examination. Suspected cancer is running about 10 per 1000 man year or higher than expected. Resection of these lesions have been done successfully. It is too soon to evaluate the project further. It seems to be progressing satisfactorily. Studies continue on the use of hematoporphyrin in the localization of tumor by bronchoscopy using a light with the proper wave length.

Significance for Cancer Research (NCP Objective X Approach)

Project Officer: Kenneth B. Olson, M.D.

Program: Lung Cancer Diagnosis

Site Visit Date:

Technical Review Group: Diagnostic Research Advisory Group

Relevance Review Group: Institute Director and Executive Committee

FY73 Funds: \$367,727

CONTRACT RESEARCH SUMMARY

Title: Determination of Tantalum Dust Properties and Development of
Tantalum Dust Delivery System for Bronchography

Principal Investigator: Dr. Morton Corn

Name/Address

Performing

Organization: University of Pittsburgh, Pittsburgh, Pa., Graduate School
of Public Health

Contract Number: 72-3229

Starting Date: March 1, 1972

Expiration Date: February 28, 1974

Goal: 1) Determine the physical properties required in tantalum dust for use
in bronchography (particle size, specific surface, density, morphology,
impurities). 2) Develop a prototype dust delivery system for use in
bronchography.

Approach: Commercially available tantalum powder is fractionated aero-
dynamically. The fractions are characterized as to particle
size, specific surface, density, shape and chemical composition.
Samples of characterized dust fractions will be provided to
other investigators for animal and human testing. A dust
generator will be designed and built to provide, within the
parameters of dust and environmental characteristics found
optimal, for use by others in animal and human testing.

Progress: Tantalum dust fractions have been separated, characterized and
made available to investigators at Johns Hopkins and the
University of California at San Francisco. A prototype dust
generator has been built and is being calibrated.

Significance for Cancer Research (NCP Objective 5 Approach 4)

The successful development of tantalum dust bronchography will
provide a vast improvement in methods for diagnostic localization
of cancer of the lung.

Project Officer: Dr. I. C. Plough

Program: Diagnosis

Site Visit Date: January 31, 1973

Technical Review Group: Ad Hoc

Relevance Review Group: DCBD Contract Review Board

FY73 Funds: \$27,000

CONTRACT RESEARCH SUMMARY

Title: "Carcinoembryonic Antigen (CEA) Test, A New Method and Its Evaluation"

Principal Investigator: Vay Liang W. Go, M.D.

Name/Address Mayo Clinic

Performing Rochester, Minnesota

Organization:

Contract Number: NIH-NCI-G-72-3854

Starting Date: June 15, 1972

Expiration Date: June 14, 1974

Goal: To evaluate the carcinoembryonic antigen test in the early diagnosis of carcinoma of the large bowel.

Approach: To develop a new and simpler method of quantitating the blood CEA level and a comparison of these findings with the Hansen test since the Hansen anti sera was found to be the most sensitive as compared to anti sera from Drs. Gold, Todd, Turner, and Posevart. This test will be used on blood from several hundred cases of gastro-intestinal cancer, lung cancer, normal subjects and others who will have non-cancerous diseases such as inflammatory bowel disease, cirrhosis etc.

Progress: The assay method appears reliable and compares favorably with the Hansen assay. In seven months 3000 assays have been done on 2100 patients and healthy subjects. The upper limit of normal has been set at 2.5 ng/ml since none of 150 subjects free of disease and only 1 of 50 heavy smokers had levels higher than this figure. All assays are not yet completed, but patients with the following conditions have had the examination completed:

Colorectal Cancer	312	26% of Duke's A, positive(20)
Cancer of the Pancreas	60	(5 islet cell tumors)
Lung Cancer	96	
Benign Conditions	over 800	

CEA is not a reliable test for small or Duke A cancer. It is positive in a substantial number of non-cancerous conditions. Study to be extended to CEA in bowel lumen.

Significance for Cancer Research (NCP Objective X Approach)

Project Officer: Kenneth B. Olson, M.D.

Program: Lung Cancer Diagnosis

Site Visit Date:

Technical Review Group: Diagnostic Research Advisory Group

Relevance Review Group: Institute Director and Executive Committee

FY73 Funds:

CONTRACT RESEARCH SUMMARY

Title: Study of Intrabronchial Deposition of Inhaled Radioopaque Particles

Principal Investigator: Morton Lippmann, Ph.D.
Name/Address Institute of Environmental Medicine
Performing New York University Medical Center
Organization: 550 First Ave.
New York, N.Y., 10016

Contract Number: 73-3855

Starting Date: November, 1972

Expiration Date: 1 year

Goal: To determine the amount of tantalum dust that must be deposited for satisfactory bronchography.

Approach: Tantalum will be deposited in various ways on the surfaces of hollow casts of the human bronchial tree, and by x-rays of this model and other methods. The exact amount of metal for good bronchograms will be determined. The model will also be used to study rates of tantalum deposition and distribution from dust clouds, as related to airway dynamics, positioning, and so forth. New dust generators will be tested as they become available.

Progress: New contract

Significance for Cancer Research (NCP Objective 5 Approach 4)

The successful development of tantalum dust bronchography will provide a vast improvement in methods for diagnosing localization of cancer of the lung.

Project Officer: Dr. I. C. Plough

Program: Diagnosis

Site Visit Date: -

Technical Review Group: Diagnosis and Miscellaneous Contracts Review Group

Relevance Review Group: DCBD Contract Review Board

FY73 Funds: \$30,159

CONTRACT RESEARCH SUMMARY

Title: "Effect of Early Detection and Treatment on Survival from Lung Cancer"

Principal Investigator: Myron R. Melamed, M.D.

Name/Address Memorial Hospital for Cancer and Allied Diseases

Performing 444 East 68th Street

Organization: New York, New York

Contract Number:

Starting Date:

Expiration Date:

Goal: To reduce the five (5) year mortality of lung cancer by 50 per cent.

Approach: This contract will add numbers to the Johns Hopkins Lung Cancer Control project and in this project there will be 2000 persons a year studied by annual x-ray of the chest and tri-yearly sputum examinations (once a year on additional aerosol sputum) as compared to a control group receiving yearly chest x-ray studies for detection of lung cancer. Cases suspicious of or positive for lung cancer by sputum cytology will have fiber optic bronchoscopy for tumor localization and hopefully less than total pneumonectomy for tumor treatment. Patients at high risk (i.e., cigarette smokers) 45 to 69 years of age will be studied. All clinical studies will be augmented by careful epidemiological surveillance. This study will be comparable to a similar and simultaneous study at Johns Hopkins University Medical School and both studies should produce statistically significant results (or lack thereof) in about five years.

Progress: Too soon to draw conclusions (new contract

Significance for Cancer Research (NCP Objective X Approach)

Project Officer: Kenneth B. Olson, M.D.

Program: Diagnosis Program

Site Visit Date: April 5, 1973

Technical Review Group: Diagnostic Research Advisory Group

Relevance Review Group: Director of Institute and Executive Committee

FY73 Funds:

SUMMARY REPORT
CYTOLOGY AUTOMATION CONTRACT PROGRAM
July 1, 1972 through June 30, 1973

The Committee on Cytology Automation was established in July, 1972 to advise the Director, DCBD, NCI on all matters pertaining to Cytology Automation. Specifically, the Committee 1) serves as advisers to NCI on the state of the art in scientific and technical areas which bear on automation, and 2) advises the NCI on plans and implementation in operational terms for NCI's contractual support of developing automation in cytopathology.

Members of the Committee are:

Dr. Chester J. Herman (Chairman)	Dr. Stanley F. Patten, Jr.
Dr. Norman G. Anderson	Mr. William E. Siri
Dr. Eugene P. Cronkite	Dr. Marvin Van Dilla
Dr. Lewis E. Lipkin	Dr. Willard H. Wattenburg
Dr. Richard A. Malmgren	Dr. George Wied
Dr. Myron R. Melamed	Mrs. Judith Prewitt
Dr. Mortimer L. Mendelsohn	

During FY 1973 4 meetings have been held:

- 1) August 9-10, 1972, Saxton's River, Vermont
- 2) November 17, 1972, NIH Reservation
- 3) January 15, 1973, NIH Reservation
- 4) March 12-13, 1973, NIH Reservation

The Committee determined that 6 areas are within the mission of the Committee:

- 1) Specimen Collection
- 2) Specimen Preparation
- 3) Sensing-screening Systems
- 4) Decision-diagnostic Systems
- 5) Specimen Sources
- 6) System Evaluation

An ongoing contract with Los Alamos Scientific Laboratory for design, fabrication and preliminary testing of a multiparameter sorter for separating abnormal cells from cytopathologic specimens falls into Category 3 (Sensing-screening Systems). This contract entered its third year in April, 1973. Collaboration with the Laboratory of Pathology, NCI on clinical evaluation of this multiparameter sorter falls generally into Category 6 (System Evaluation).

A contract was begun in April, 1973 with the University of Chicago for application of a high-resolution image-processing system (TICAS) to clinical cytopathologic material. This contract falls into Category 4 (Decision-diagnostic systems).

A third contract approved and signed in April, 1973 bridges Categories 3 and 4. This contract with the University of Rochester is for the application of a slit-scan, medium-resolution sensing system to cells in a rapid-flow suspension.

Six Requests for Proposals have been formulated by the Committee and are being published. The first of these call for a retrospective comparison of various types of cytopathologic specimens with biopsies to determine the composition of clinical specimens and the optimal diagnostic specimens for routine use. This information is critically necessary to determine the constraints which apply to all automated systems.

Two RFP's are being published requesting proposals for new methods for preparing monodisperse cell samples in solution and on slides.

Three RFP's are being published for development of new markers for cells. Such markers include new dyes, both absorption and fluorescent, for cells both in suspension and on slides. In addition, new biochemical and biophysical methods of interrogating cells are being sought. Such new methods, of proven value in research applications and of great potential value and power in clinical cytopathology, would include centrifugation, sedimentation, holography, acoustic techniques, etc.

Projects in these three areas are the immediate priority of the Committee.

CONTRACT RESEARCH SUMMARY

Title: Automated Cancer-Cell Sorting and Analysis

Principal Investigator: Dr. Paul F. Mullaney
Name/Address U.S. Atomic Energy Commission
Performing Los Alamos Scientific Laboratory
Organization: Los Alamos, New Mexico 87544

Contract Number: NIH-NCI-GLC-(71)-55

Starting Date: February 12, 1971

Expiration Date: March 31, 1974

Goal: Automation of clinical cytologic screening and diagnosis.

Approach: A machine will be developed and tested by the contractor to measure and analyze cytobiological parameters related to malignancy. This machine will separate cells and specimens suggestive of malignancy for subsequent definitive diagnosis. During development of the sorting machine, those cytobiological parameters most reliably related to separation of malignant from non-malignant cells and those techniques of sample preparation most suitable for supporting machine analysis will be identified by collaborative work in the contractor's laboratory and in the NCI Laboratory of Pathology. When these phases of the work are completed, the machine, the parameters and the associated sample preparation techniques will be tested on clinical cytologic material.

Progress: To date preliminary investigation has indicated that tumor cells can be separated from hematopoietic and squamous cells. A second multiparameter sorter for intensive biological use at the contractor's facility is being bench-tested. A third multiparameter sorter for investigation of clinical cytologic material at NCI is under construction.

Significance for Cancer Research (NCP Objective 5 Approach 5.3.) The use of a very rapid zero-resolution automated screening system in clinical cytodiagnosis will allow greater availability of these services to a much larger population with improved diagnostic accuracy.

Project Officer: Chester J. Herman, M.D.

Program: Cytology Automation **Site Visit Date:** January 18, 1973

Technical Review Group: Committee on Cytology Automation

Relevance Review Group: DCBD Contract Review Board

FY73 Funds: \$256,500

CONTRACT RESEARCH SUMMARY

Title: Investigation of a Slit-Scan Technique as a Basis for an Automated Prescreening System for Cancer Detection in Cytology

Principal Investigator: Leon L. Wheelless, Jr., Ph.D.

Name/Address University of Rochester

Performing School of Medicine and Dentistry

Organization: Rochester, New York 14620

Contract Number: NIH-NCI-1-CB-33862

Starting Date: March 19, 1973

Expiration Date: March 18, 1974

Goal: Automation of clinical cytologic screening and diagnosis.

Approach: The investigator has made extensive use of the slit-scan technique to investigate cytologic specimens on slides. Use of acridine orange fluorochrome with this system allows good separation of signals of pre-malignant and malignant cells from those of non-malignant cells. Work under the present contract will be directed at adaptation of the slit-scan technique to a rapid flow system. The use of this flow system with medium resolution scanning on clinical specimens will be evaluated.

Progress: The contract has just begun.

Significance for Cancer Research (NCP Objective 5 Approach 5.4) A medium-resolution flow system for automating clinical cytology will allow greater accuracy in screening and diagnosis of cancerous and pre-cancerous lesions as well as increasing speed of the technique and wider availability to a larger population.

Project Officer: Chester J. Herman, M.D.

Program: Cytology Automation

Site Visit Date:

Technical Review Group: Committee on Cytology Automation

Relevance Review Group: DCBD Contract Review Board

FY73 Funds: \$151,347

CONTRACT RESEARCH SUMMARY

Title: Evaluation of Information Requirements for Image Processing of Cell Samples as a Basis for Development of an Automated Cell Recognition System.

Principal Investigator: George L. Wied, M.D.
Name/Address: The University of Chicago
Performing: 5841 Maryland Avenue
Organization: Chicago, Illinois 60637

Contract Number: NIH-NCI-1-CB-33873

Starting Date: April 23, 1973

Expiration Date: April 22, 1974

Goal: Automation of clinical cytologic screening and diagnosis.

Approach: The principal investigator's TICAS (Taxonomonic Intracellular Analytic System) has proved very valuable in obtaining sophisticated analyses of the morphologic properties of cells of a number of histologic types. Under this contract, the application of TICAS to gynecologic cervical specimens on smears will be investigated.

Progress: The contract has just begun.

Significance for Cancer Research (NCP Objective⁵ Approach^{5.4}) The use of sophisticated image analysis will produce greater diagnostic accuracy on cytologic specimens than is presently available from visual inspection of smears.

Project Officer: Judith Prewitt (Dr. Chester Herman)

Program: Cytology Automation

Site Visit Date: December 20, 1972

Technical Review Group: Committee on Cytology Automation

Relevance Review Group: DCBD Contract Review Board

FY73 Funds: \$187,421

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